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FIGURE 1

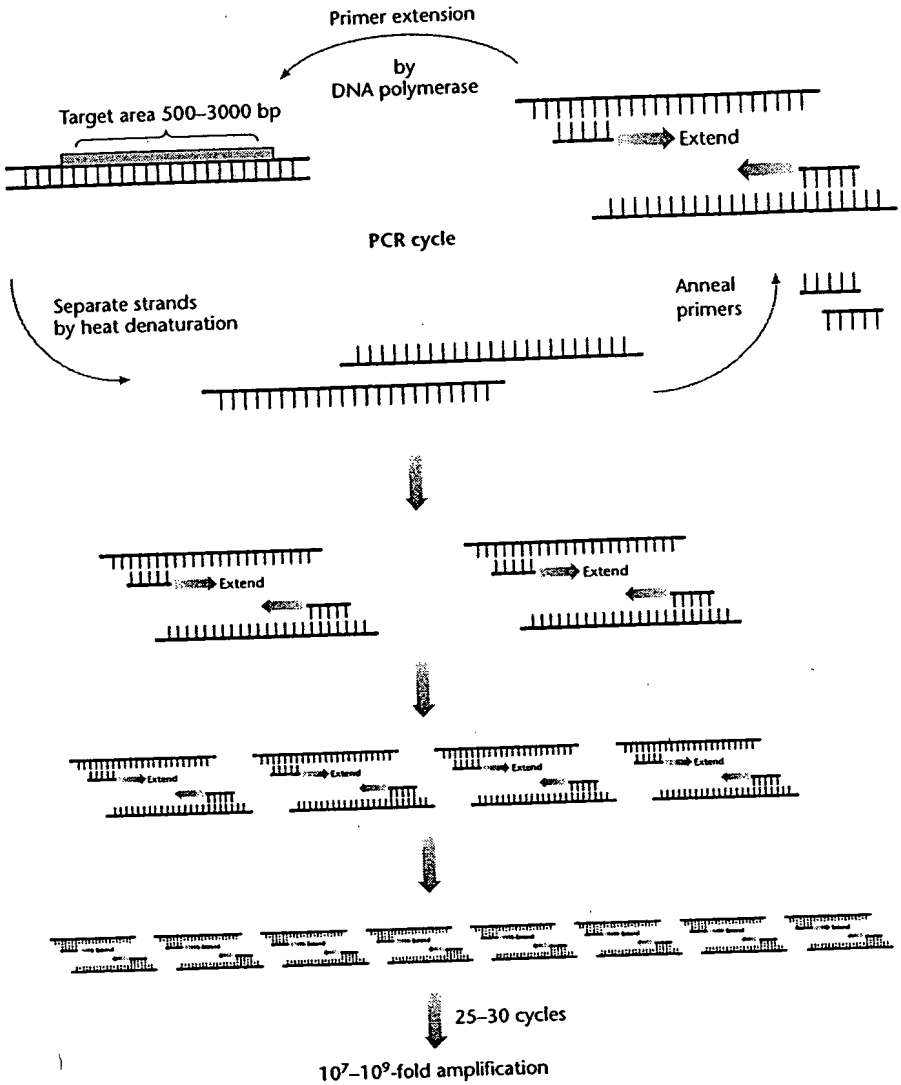

Figure 1 The PCR amplification cycle.

primer sequences, but manual inspection of the oligonucleotide is still necessary to maximize successful PCR amplifications.

The concentrations of the PCR cocktail ingredients are also important for product specificity, fidelity and yield. In addition to *Taq* DNA polymerase and primers, the PCR mixture contains the cofactor magnesium ion (Mg^{2+}), the four 2′-deoxyribonucleoside-5′-triphosphates (dNTPs) and the buffer. In general, PCR reagent concentrations that are too high from 'standard conditions' result in nonspecific products with high misincorporation errors, and those that are too low result in insufficient product. A typical 50-µl PCR cocktail that contains 0.4 µmol L^{-1} of each primer, 200 µmol L^{-1} of each dNTP, 1.5 mmol L^{-1} $MgCl_2$, and 1.25 units *Taq* DNA polymerase in 10 mmol L^{-1} tris-HCl, pH 8.3, 50 mmol L^{-1} KCl buffer works well for most PCR applications. The optimal Mg^{2+} concentration, however, may need to be determined empirically for difficult target templates. The performance and fidelity of *Taq* DNA polymerase are sensitive to the free Mg^{2+} concentration (Eckert and Kunkel, 1990), which ionically interacts with not only the dNTPs but also with the primers, the template DNA, ethylenediaminetetraacetic acid (EDTA), and other chelating agents. In most cases, the Mg^{2+} concentration will range from 1.0 to 4.0 mmol L^{-1}.

The number of cycles and the cycle temperature/length of time for template denaturation and primer annealing and extension are important parameters for high-quality PCR results. The optimal number of cycles is dependent on

(Metzker ML et al.)

ANNEX I (P150→P156

17

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF ANGIOTENSIN II AT2 RECEPTOR IN NEUROBLASTOMA N1E-115 CELLS

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INTRODUCTION

Since the discovery in 1989 of a second subtype (AT2) of angiotensin II (Ang II) receptors (1, 2), its functional significance has remained a matter of debate. Expression of AT2 receptors was detected in adrenals, uterus, ovary, heart, brain, and at highest levels in the developing fetus. This subtype has been associated with reduction of intracellular cGMP levels (3-5), modulation of tyrosine phosphorylation (4, 6-9), inhibition of a T-type calcium channel (10, 11), opening of potassium channels (12, 13), release of prostaglandin (14) and production of arachidonic acid (15). Recent molecular cloning of the AT2 receptor cDNA in rat (6, 16), mouse (17) and human (18, 19) revealed a structural organization typical of G-protein coupled receptors, but did not provide yet a clear understanding of transduction pathways and physiological functions associated with this subtype.

To address the question of AT2 signaling, we have undertaken a detailed pharmacological, molecular and functional analysis of this receptor subtype in the murine neuroblastoma cell line N1E-115 (9). These cells express a single molecular class of AT2 receptors and no detectable AT1 binding site. In these cells, AT2 receptors are functionally coupled to protein tyrosine dephosphorylation. The N1E-115 cell line thus constitutes a simple and well-characterized cellular model for further analyses of AT2 receptor regulation and function.

PHARMACOLOGICAL CHARACTERIZATION OF ANG II RECEPTORS IN N115 CELLS

Subtypes (AT1 and AT2) of Ang II receptors expressed in neuroblastoma N1E-115 cells were analyzed using the non-selective iodinated Ang II antagonist : ^{125}I-(Sar, Ile)-AngII,

Recent Advances in Cellular and Molecular Aspects of Angiotensin Receptors
Edited by Mohan K. Raizada et al., Plenum Press, New York, 1996

Table I. Binding parameters of various ligands to membranes of N1E-115 cells and COS-AT2 cells. Values are from Nahmias et al. (9). n.d. stands for non determined

	N1E-115		COS-AT2	
Radioligand	Kd(pM)	Bmax(fmol/mg)	Kd(pM)	Bmax(fmol/mg)
^{125}I-(Sar,Ile)AngII	233±33	298±53	n.d.	n.d.
^{125}I-CGP 42112	91±19	321±42	75±4	125±11
Competitor	IC50 (nM)		IC50 (nM)	
(Sar,Ile)AngII	0.45±0.09		0.39±0.07	
CGP 42112	0.53±0.15		0.45±0.08	
Ang II	1.22±0.12		0.84±0.28	
PD 123319	4.60±0.21		4.74±0.55	
DUP 753	> 10,000		>10.000	

and the AT2-selective radioligand ^{125}I-CGP 42112. Scatchard analyses revealed that both ligands bound to a single class of high affinity binding sites with the same Bmax (Table I), indicating that most if not all Ang II receptors were of the AT2 subtype. The absence of the AT1 subtype was confirmed by the inefficiency of the AT1-selective ligand DUP 753 to compete for binding of ^{125}I-(Sar, Ile)-AngII (Table I). In contrast, the AT2-selective ligands CGP 42112 and PD 123319 were able to totally displace the binding of ^{125}I-(Sar, Ile)-AngII in monophasic curves, indicating the presence of a single pharmacological class of AT2 receptors, which binding parameters are presented in Table I.

Fluharty and colleagues (20, 21) previously reported expression of both AT1 and AT2 receptor subtypes in N1E-115 cells. More recently, these authors identified two subpopulations of AT2 receptors (designated as peak I and peak III, respectively) that could be separated by heparine sepharose chromatography (22). These two populations differed in their affinity for the AT2-selective ligand PD 123319, their sensitivity to DTT and to analogs of GTP, and their reactivity towards a polyclonal anti-AT2 receptor antiserum. Discrepancy between these data and ours may be explained by the divergence occuring in cell lines that were independently passaged for many years under different culture conditions. The AT2 subtype expressed in our cell line shows moderate affinity for PD 123319, increased binding in the presence of reducing reagent and insensitivity to GTPgS (9), and would thus correspond to the so-called "peak III".

Cloning of AT2 receptor cDNA from N1E-115

For a further molecular characterization, cDNA clones were isolated from a N1E-115 cDNA library by homology screening, using a DNA probe derived by a PCR strategy on the basis of the rat AT2 receptor sequence (9). Nucleotide sequencing revealed that AT2 cDNA clones from N1E-115 belong to the same molecular subtype as those isolated from rat (16) and mouse (17) fetal tissues, as well as from rat pheochromocytoma PC12W cells (6), human myometrium (18) and human lung (19).

One remarkable feature of the mouse AT2 cDNA sequence is the presence of two in-frame initiator ATG codons that potentially generate two polypeptides of different length (Fig.1). The downstream ATG codon is more likely utilized, as its position is in agreement with Kozak's consensus for initiation of translation (23), and corresponds to the single initiator ATG codon present in the rat (6, 16) and the human (18, 19) AT2 sequences. It cannot be excluded however that in certain conditions, translation is initiated at the upstream ATG

codon, leading to expression of a polypeptide carrying 30 additional amino-terminal residues. Deletion of the cDNA sequence corresponding to these 30 amino acids did not affect the binding properties of the receptor expressed in COS cells (data not shown). Translation of these additional residues may nevertheless affect the stability or the routing of the molecule inside the cell.

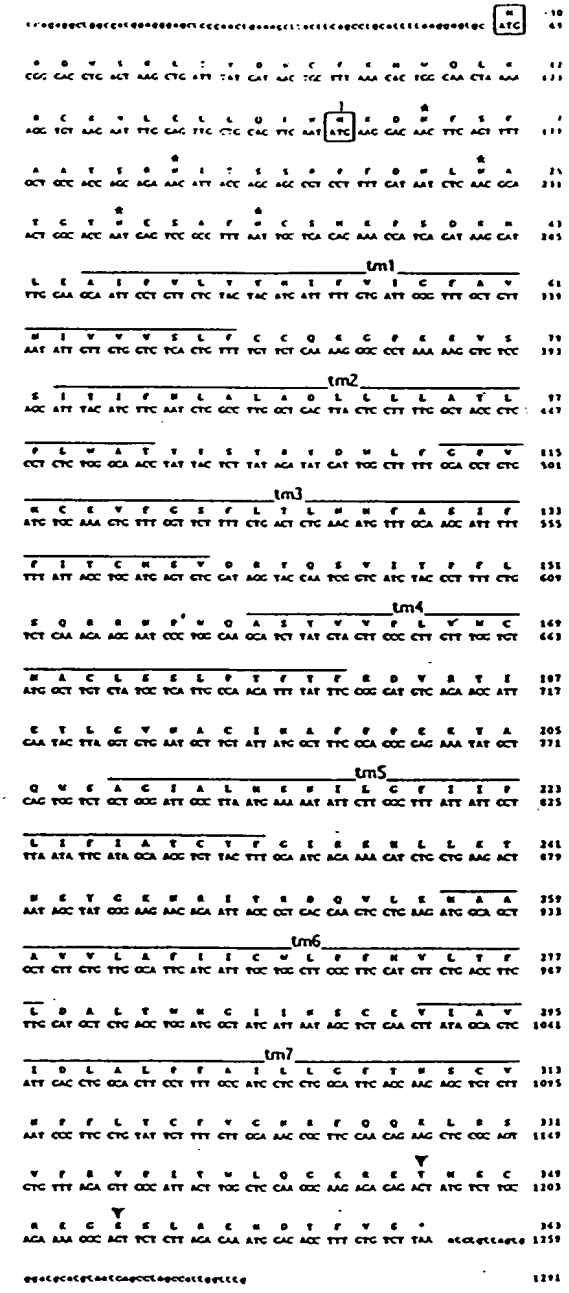

Figure 1. Nucleotide and deduced amino-acid sequences of the AT2 cDNA from N1E-115 cells. Two potential initiator ATG codons are boxed. Amino-acid number one corresponds to the downstream initiator ATG codon (position 157 of the nucleotide sequence). The seven transmembrane segments (tm1-tm7) are highlighted by solid bars. Potential N-linked glycosylation sites are indicated by stars. Putative sites for phosphorylation by protein kinase A and protein kinase C are indicated by triangles.

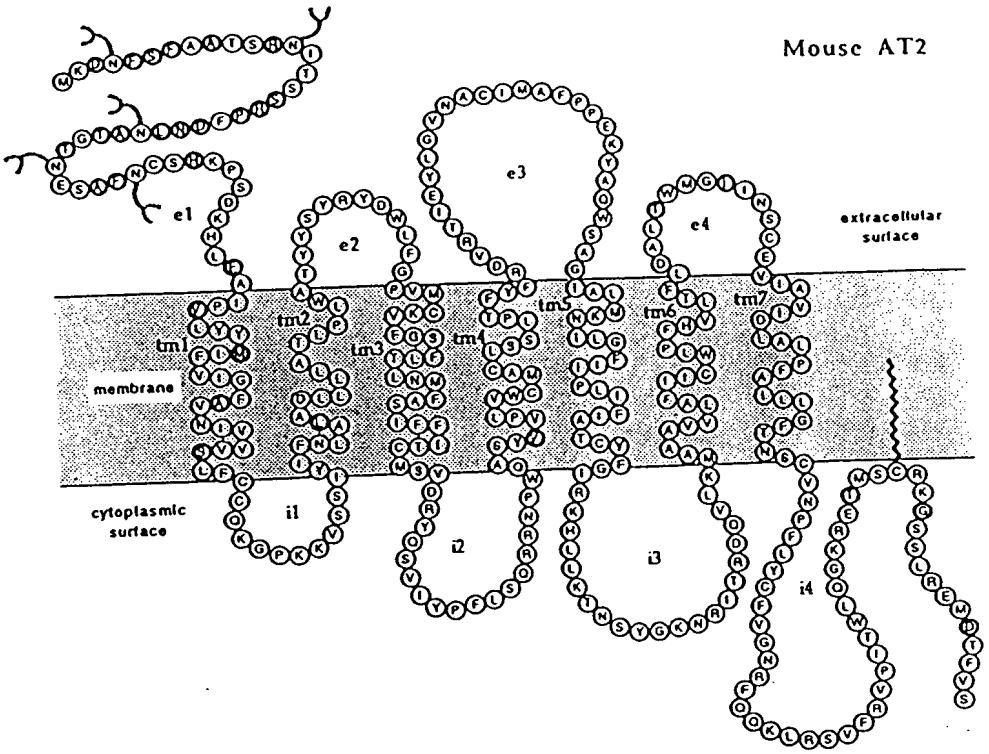

Figure 2. Putative organization of the murine AT2 receptor within the cell membrane. Five potential N-linked glycosylated sites in the amino-terminus and a putative palmitylated cysteine in the carboxy-terminus are indicated. Residues which differ between mouse and human AT2 sequences are in bold.

The deduced amino-acid sequence of the mouse AT2 polypeptide (Fig. 2) displays all the features characteristic of G-protein coupled receptors (24), including the presence of seven membrane-spanning domains, potential N-linked glycosylation sites, and consensus sites for phosphorylation by protein kinases in the carboxy terminal tail. Sequence comparison indicates high conservation (92% amino-acid sequence homology) between human and murine AT2 receptors (Fig. 2). Most divergent residues are clustered in the extracellular, N-terminal portion of the molecule. Despite these differences, five putative N-linked glycosylation sites are conserved at identical positions in the amino-terminal part of the AT2 receptor in both species.

The isolated cDNA clones were functionally expressed in COS cells, yielding pharmacological parameters identical to those of endogeneous Ang II receptors from N1E-115 cells (Table I). This confirms that N1E-115 cells express exclusively the AT2 receptor subtype, and identifies this receptor entity at the molecular level.

FUNCTIONAL STUDIES OF AT2 RECEPTORS: COUPLING TO PROTEIN TYROSINE DEPHOSPHORYLATION

The pharmacological and molecular identification of a single class of AT2 receptors in N1E-115 cells prompted us to further analyze this receptor subtype at the functional level.

The relationship between AT2 signaling and intracellular protein tyrosine phosphorylation was investigated by Western blot analysis of total cell lysates using anti-phosphotyrosine antibodies. Treatment of N1E-115 cells with Ang II led to a reduction in tyrosine phosphorylation of several endogeneous proteins, of apparent molecular masses 80, 97, 120, 150 and 180 kDa, respectively (Fig. 3). This effect was rapid and transient, showing a maximum at 5 to 10 minutes and declining at 30 minutes. This response could be blocked in the presence of an excess of the antagonist (Sar, Ile)-Ang II and was also obtained following treatment with the AT2-selective agonist CGP 42112 (9).

Preliminary studies of Chinese Hamster Ovary (CHO) cells stably expressing the mouse AT2 receptor indicated that in this cellular model, AT2 stimulation also results in the transient dephosphorylation of cellular proteins on tyrosine residues (data not shown). The same intracellular effect could not however be detected in COS cells transiently transfected with either the murine or the human AT2 receptor cDNA. This may indicate that COS cells lack one or several components of the AT2 signaling pathway. It is worth noting that other groups reporting the cloning of AT2 receptor cDNA failed to detect any intracellular effect of this receptor after both transient (16) and stable (6) overexpression in COS cells.

Our results showing a reduction of intracellular protein tyrosine phosphorylation may be explained by stimulation of a protein tyrosine phosphatase (PTP) activity, or by inhibition of a tyrosine kinase-mediated pathway. Given the complexity of intracellular cascades of kinases and phosphatases (25-27), it is indeed possible that AT2 receptors differentially affect several types of enzymes acting at different levels. In the PC12W cell line, AT2 receptors have been reported to stimulate a PTP activity by a G-protein-independent mechanism (4, 7) and to inhibit a PTP through a pertussis toxin-mediated pathway (6, 8). Progress in understanding AT2 signaling will greatly benefit from investigating molecular determinants of AT2-mediated pathway, including identification of the G-protein(s) involved, search for cellular "interaction partners" of the receptor, elucidation of PTPs/TKs activities modulated by the AT2 subtype, and identification of endogeneous phosphoproteins which are dephosphorylated following AT2 receptor activation.

The relationship between AT2 receptors and tyrosine phosphorylation suggests a link with physiological processes such as cell growth, differentiation or cellular adhesion, which are known to involve cascades of protein kinases and phosphatases (28). Possible role for AT2 receptors in these processes is also consistent with increased levels of expression of this subtype during embryonic development (29) and in pathological situations such as vascular growth (30) and tissue repair (31). In vivo studies have indeed identified AT2 receptors as

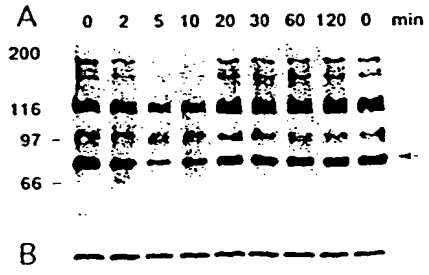

Figure 3. Ang II-induced tyrosine dephosphorylation in N1E-115 cells. (A) N1E-115 cells were incubated with Ang II for various periods of time, before lysis and subsequent immunoblotting with anti-phosphotyrosine antibodies. On the left are indicated the positions of standard molecular weight markers, and the arrow on the right shows the position of a major phosphoprotein of 80 kDa. (B) After removal of bound antibodies, the same blot was incubated with anti-ezrin antibodies for internal control of the amount of proteins in each lane.

participating in vascular neointima formation (30) and angiogenesis (32). More recently, anti-proliferative effects of AT2 receptors in primary cultures of rat endothelial coronary cells have been demonstrated (33). Other studies performed in cultured cells tend to indicate, however, inverse relationship between expression of AT2 receptors and cell proliferation (34-36).

ARE THERE MULTIPLE SUBCLASSES OF AT2 RECEPTORS?

Studies conducted in the rat brain have pointed out possible heterogeneity of AT2 receptors. Quantitative autoradiography revealed differential sensitivity of CGP 42112-binding sites to analogs of GTP (37) and to reducing reagents (38, 39). In addition, immunohistochemical staining of rat brain sections using anti-AT2 polyclonal antibodies correlated with, but was not identical to patterns of radioligand binding (40). As mentioned before, two pharmacologically, biochemically and immunologically distinct populations of AT2 receptors could be identified in N1E-115 cells (22). These observations, as well as the diverse and sometimes contradictory reports of AT2 signaling, may be due to expression of a single molecular entity in different cell environments, and/or may reflect the existence of multiple subclasses of AT2 receptors.

The AT2 receptor gene exists as a single copy in the genome of both human (18) and mouse (17). This argues against the possibility that several highly homologous AT2 genes exist, as is the case for rodent AT1a and AT1b. In addition, the coding region of the AT2 gene is devoid of introns, thus ruling out the hypothesis that alternative splicing may lead to multiple forms of receptors. It remains possible however that "AT2 receptors" in fact consist of several molecular entitites, that do not share high amino-acid sequence homology but that all exhibit high affinity for ligands such as CGP 42112 and PD 123319 which define the AT2 subtype pharmacologically.

Further characterization of AT2 receptors in a variety of cellular environments is now made possible by the development of series of new ligands (41, 42) and may help discriminate between putative AT2 receptor subtypes. The molecular basis of AT2 receptor heterogeneity will also certainly be investigated in the coming years, as more and more AT2 cDNA clones will be isolated from different tissues and cell lines.

ACKNOWLEDGMENTS

This work was supported by the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, University Paris VII, and the Ministry for Research. We are also grateful to the Association pour la Recherche sur le Cancer, the Fondation pour la Recherche Medicale and the Ligue Nationale contre le Cancer.

REFERENCES

1. Chiu, A. T. *et al.* (1989). Eur J Pharmacol *170*: 117-118.
2. Whitebread, S., Mele, M., Kamber, B. and de Gasparo, M. (1989). Biochem Biophys Res Commun *163*: 284-291.
3. Sumners, C., Tang, W., Zelezna, B. and Raizada, M. K. (1991). Proc Natl Acad Sci U S A *88*: 7567-7571.
4. Bottari, S. P., King, I. N., Reichlin, S., Dahlstroem, I., Lydon, N. and de Gasparo, M. (1992). Biochem Biophys Res Commun *183*: 206-211.
5. Brechler, V., Levens, N. R., de Gasparo, M. and Bottari, S. P. (1993). Regulatory Peptides *44*: 207-213.

6. Kambayashi, Y., Bardhan, S., Takahashi, K., Tsuzuki, S., Inui, H., Hamakubo, T. and Inagami, T. (1993). J Biol Chem *268*: 24543-24546.
7. Brechler, V., Reichlin, S., de Gasparo, M. and Bottari, S. P. (1994). Receptors and Channels *2*: 89-97.
8. Takahashi, K., Bardhan, S., Kambayashi, Y., Shirai, H. and Inagami, T. (1994). Biochem Biophys Res Commun *198*: 60-66.
9. Nahmias, C., Cazaubon, S. M., Briend-Sutren, M.-M., Lazard, D., Villageois, P. and Strosberg, A. D. (1995). Biochem J *306*: 87-92.
10. Buisson, B., Bottari, S. P., de Gasparo, M., Gallo, P. N. and Payet, M. D. (1992). FEBS lett. *309*: 161-164.
11. Buisson, B., Laflamme, L., Bottari, S. P., de Gasparo, M., Gallo-Payet, N. and Payet, M. D. (1995). J Biol Chem *270*: 1670-1674.
12. Kang, J., Posner, P. and Sumners, C. (1994). Am J Physiol *267*: C1289- C1397.
13. Sumners, C., Raizada, M. K., Kang, J., Lu, D. and Posner, P. (1994). Frontiers in Neuroendocrinology *15*: 203-230.
14. Jaiswal, N., Tallant, E. A., Diz, D. I., Khosla, M. C. and Ferrario, C. M. (1991). Hypertension *17*: 1115-1120.
15. Lokuta, A. J., Cooper, C., Gaa, S. T., Wang, H. E. and Rogers, T. B. (1994). J Biol Chem *269*: 4832-4838.
16. Mukoyama, M., Nakajima, M., Horiuchi, M., Sasamura, H., Pratt, R. E. and Dzau, V. J. (1993). J Biol Chem *268*: 24539-24542.
17. Nakajima, M., Mukoyama, M., Pratt, R. E., Horiuchi, M. and Dzau, V. J. (1993). Biochem Biophys Res Commun *197*: 393-399.
18. Lazard, D., Briend-Sutren, M.-M., Villageois, P., Mattei, M.-G., Strosberg, A. D. and Nahmias, C. (1994). Receptors and Channels *2*: 271-280.
19. Martin, M. M., Su, B. G. and Elton, T. S. (1994). Biochem Biophys Res Commun *205*: 645-651.
20. Reagan, L. P., Ye, X., Mir, R., DePalo, L. R. and Fluharty, S. J. (1990). Mol Pharmacol *38*: 878-886.
21. Reagan, L. P., Ye, X., Maretzski, C. H. and Fluharty, S. J. (1993). J Neurochem *60*: 24-31.
22. Siemens, I. R., Reagan, L. P., Yee, D. K. and Fluharty, S. J. (1994). J Neurochem *62*: 2106-2115.
23. Kozak, M. (1987). Nuc Acids Res *15*: 8125-8132.
24. Strosberg, A. D. (1991). Eur J Biochem *196*: 1-10.
25. Vogel, W., Lammers, R., Huang, J. and Ullrich, A. (1993). Science *259*: 1611-1614.
26. Sun, H. and Tonks, N. K. (1994). Trends Biochem. Sci. *19*: 480-485.
27. Hunter, T. (1995). Cell *80*: 225-236.
28. Schlessinger, J. and Ullrich, A. (1992). Neuron *9*: 383-391.
29. Grady, E. F., Sechi, L. A., Griffin, C. A., Schambelan, M. and Kalinyak, J. E. (1991). J Clin Invest *88*: 921-933.
30. Janiak, P., Pillon, A., Prost, J. F. and Vilaine, J. P. (1992). Hypertension *20*: 737-745.
31. Viswanathan, M. and Saavedra, J. M. (1992). Peptides *13*: 783-786.
32. le Noble, F. A. C., Schreurs, N., van Straaten, H. W. M., Slaaf, D. W., Smits, J. F. M. and Struyker Boudier, H. A. J. (1992). FASEB J *6*: A937 (Abstract).
33. Stoll, M., Steckelings, M., Paul, M., Bottari, S. P., Metzger, R. and Unger, T. (1995). J Clin Invest *95*: 651-657.
34. Dudley, D. T. and Summerfelt, R. M. (1993). Regul Pept *44*: 199-206.
35. Grady, E. F. and Kalinyak, J. E. (1993). Regul Pept *44*: 171-80.
36. Kambayashi, Y., Bardhan, S. and Inagami, T. (1993). Biochem Biophys Res Commun *194*: 478-82.
37. Tsutsumi, K. and Saavedra, J. M. (1992). Mol Pharmacol *41*: 290-297.
38. Tsutsumi, K., Zorad, S. and Saavedra, J. M. (1992). Eur. J. Pharmacol. *226*: 169-173.
39. Speth, R. C. (1993). Regulatory peptides *44*: 189-197.
40. Reagan, L. P., Flanagan-Cato, L. M., Yee, D. K., Ma, L.-Y., Sakai, R. R. and Fluharty, S. J. (1994). Brain Res. *662*: 45-59.
41. VanAtten, M. K., Ensinger, C. L., Chiu, A. T., McCall, D. E., Nguyen, T. T., Wexler, R. R. and Timmermans, P. B. (1993). J Med Chem *36*: 3985-3992.
42. Chang, L. L. *et al.* (1994). J Med Chem *37*: 4464-4478.

Human (avant dépôt (voir page 3))

ANNEX II (9 pages)

NCBI Protein

Entrez PubMed Nucleotide Protein Genome Structure PMC Taxonomy Books

Search Nucleotide for Go Clear

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Display default Show: 20 Send to File Get Subsequence Features

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)URCE       Homo sapiens (human)
 ORGANISM   Homo sapiens
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 AUTHORS    Martin,M.M. and Elton,T.S.
 TITLE      The sequence and genomic organization of the human type 2
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 TITLE      Assignment of the human angiotensin II type 2 receptor gene (AGTR2)
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 TITLE      Human type 2 angiotensin II receptor gene: cloned, mapped to the X
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Annexe II

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TITLE Molecular characterization and chromosome localization of a human angiotensin II AT2 receptor gene highly expressed in fetal tissues
JOURNAL Recept. Channels 2 (4), 271-280 (1994)
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[SUBCELLULAR LOCATION] INTEGRAL MEMBRANE PROTEIN.
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Region          72..80
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Region          81..102
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="2."
Region          103..119
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                /region_name="Domain"
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Region          120..140
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                /note="3."
Region          141..160
                /gene="AGTR2"
                /region_name="Domain"
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Region          161..179
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="4."
Region          180..208
                /gene="AGTR2"
                /region_name="Domain"
                /note="EXTRACELLULAR."
Region          209..234
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                /region_name="Transmembrane region"
                /note="5."
Region          235..256
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Region          257..278
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                /region_name="Transmembrane region"
                /note="6."
Region          268..269
                /gene="AGTR2"
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                /note="CW -> WC (IN REF. 5)."
Region          272
                /gene="AGTR2"
                /region_name="Conflict"
                /note="F -> L (IN REF. 4)."
Region          279..285
                /gene="AGTR2"
                /region_name="Domain"
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Region          286..313
                /gene="AGTR2"
                /region_name="Transmembrane region"
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Region          314..363
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                /region_name="Domain"
                /note="CYTOPLASMIC."
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                    /region_name="Conflict"
                    /note="N -> G (IN REF. 4)."
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      61 vnivvvtlfc cqkgpkkvss iyifnlavad llllatlplw atyysyrydw lfgpvmckvf
     121 gsfltlnmfa siffitcmsv dryqsviypf lsqrrnpwqa syivplvwcm aclsslptfy
     181 frdvrtieyl gvnacimafp pekyaqwsag ialmknilgf iiplifiatc yfgirkhllk
     241 tnsygknrit rdqvlkmaaa vvlafiicwl pfhvltflda lawmgvinsc eviavidlal
     301 pfaillgftn scvnpflycf vgnrfqqklr svfrvpitwl qgkresmscr kssslremet
     361 fvs
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NCBI

Sequence Revision History

Revision history for P50052

GI	Version	Update Date	Status	I	II
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1703214	n/a	Jan 20 2004 9:12 AM	Dead	○	◉
1703214	n/a	Nov 11 2003 8:21 AM	Dead	○	○
1703214	n/a	Apr 1 2002 5:32 PM	Dead	○	○
1703214	n/a	Jun 25 2001 1:17 PM	Dead	○	○
1703214	n/a	Apr 16 2001 1:28 PM	Dead	○	○
1703214	n/a	Feb 1 2001 10:23 AM	Dead	○	○
1703214	n/a	Jun 12 2000 2:54 PM	Dead	○	○
1703214	n/a	Apr 3 2000 11:14 AM	Dead	○	○
1703214	n/a	Feb 7 2000 1:29 PM	Dead	○	○
1703214	n/a	Jan 31 2000 1:20 PM	Dead	○	○
1703214	n/a	Nov 4 1999 2:07 PM	Dead	○	○
1703214	n/a	Sep 21 1999 11:25 AM	Dead	○	○
1703214	n/a	Jul 21 1998 4:10 AM	Dead	○	○
1703214	n/a	Jun 15 1998 12:21 AM	Dead	○	○
1703214	n/a	Jun 4 1998 4:07 AM	Dead	○	○
1703214	n/a	Apr 10 1998 12:54 AM	Dead	○	○
1703214	n/a	Feb 4 1998 10:19 AM	Dead	○	○
1703214	n/a	Oct 9 1997 9:39 PM	Dead	○	○
1703214	n/a	Dec 3 1996 9:38 PM	Dead	○	○

Accession P50052 was first seen at NCBI on Dec 3 1996 9:38 PM

1: P50052. Type-2 angiotensi...[gi:1703214]

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LOCUS       P50052                    363 aa            linear   PRI 15-MAR-2004
DEFINITION  Type-2 angiotensin II receptor (AT2).
ACCESSION   P50052
VERSION     P50052  GI:1703214
DBSOURCE    swissprot: locus AG22_HUMAN, accession P50052;
            class: standard.
            extra accessions:Q13016,created: Oct 1, 1996.
            sequence updated: Oct 1, 1996.
            annotation updated: Mar 15, 2004.
            xrefs: gi: 747969, gi: 747970, gi: 510700, gi: 510701, gi: 607811,
            gi: 607812, gi: 558882, gi: 558883, gi: 595934, gi: 595935, gi:
            32482004, gi: 32482005, gi: 1143833, gi: 1143834, gi: 860958, gi:
            860959, gi: 1082208
            xrefs (non-sequence databases): HSSPP34996, GenewHGNC:338, MIM
            300034, GO0005887, GO0004945, GO0004860, GO0006915, GO0007610,
            GO0007166, GO0008217, InterProIPR000276, PfamPF00001,
            PRINTSPR00237, PROSITEPS00237, PROSITEPS50262
KEYWORDS    G-protein coupled receptor; Transmembrane; Glycoprotein;
            Polymorphism.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 363)
  AUTHORS   Martin,M.M. and Elton,T.S.
  TITLE     The sequence and genomic organization of the human type 2
            angiotensin II receptor
  JOURNAL   Biochem. Biophys. Res. Commun. 209 (2), 554-562 (1995)
  MEDLINE   95251653
   PUBMED   7733925
  REMARK    SEQUENCE FROM N.A.
            TISSUE=Liver
REFERENCE   2  (residues 1 to 363)
  AUTHORS   Chassagne,C., Beatty,B.G. and Meloche,S.
  TITLE     Assignment of the human angiotensin II type 2 receptor gene (AGTR2)
            to chromosome Xq22-q23 by fluorescence in situ hybridization
  JOURNAL   Genomics 25 (2), 601-603 (1995)
  MEDLINE   95309939
   PUBMED   7790004
  REMARK    SEQUENCE FROM N.A.
            TISSUE=Placenta
REFERENCE   3  (residues 1 to 363)
  AUTHORS   Koike,G., Horiuchi,M., Yamada,T., Szpirer,C., Jacob,H.J. and
            Dzau,V.J.
  TITLE     Human type 2 angiotensin II receptor gene: cloned, mapped to the X
            chromosome, and its mRNA is expressed in the human lung
  JOURNAL   Biochem. Biophys. Res. Commun. 203 (3), 1842-1850 (1994)
  MEDLINE   95032069
   PUBMED   7945336
  REMARK    SEQUENCE FROM N.A.
            TISSUE=Blood
REFERENCE   4  (residues 1 to 363)
  AUTHORS   Tsuzuki,S., Ichiki,T., Nakakubo,H., Kitami,Y., Guo,D.F., Shirai,H.
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and Inagami,T.
TITLE Molecular cloning and expression of the gene encoding human angiotensin II type 2 receptor
JOURNAL Biochem. Biophys. Res. Commun. 200 (3), 1449-1454 (1994)
MEDLINE 94242007
PUBMED 8185599
REMARK SEQUENCE FROM N.A.
TISSUE=Placenta
EFERENCE 5 (residues 1 to 363)
AUTHORS Martin,M.M., Su,B. and Elton,T.S.
TITLE Molecular cloning of the human angiotensin II type 2 receptor cDNA
JOURNAL Biochem. Biophys. Res. Commun. 205 (1), 645-651 (1994)
MEDLINE 95091796
PUBMED 7999093
REMARK SEQUENCE FROM N.A.
TISSUE=Lung
EFERENCE 6 (residues 1 to 363)
AUTHORS Lazard,D., Briend-Sutren,M.M., Villageois,P., Mattei,M.G., Strosberg,A.D. and Nahmias,C.
TITLE Molecular characterization and chromosome localization of a human angiotensin II AT2 receptor gene highly expressed in fetal tissues
JOURNAL Recept. Channels 2 (4), 271-280 (1994)
MEDLINE 95236034
PUBMED 7719706
REMARK SEQUENCE FROM N.A.
TISSUE=Placenta
EFERENCE 7 (residues 1 to 363)
AUTHORS Kopatz,S.A., Aronstam,R.S. and Sharma,S.V.
TITLE Direct Submission
JOURNAL Submitted (~JUN-2003)
REMARK SEQUENCE FROM N.A.
EFERENCE 8 (residues 1 to 363)
AUTHORS Katsuya,T. and Dzau,V.J.
TITLE Direct Submission
JOURNAL Submitted (~JAN-1996)
REMARK SEQUENCE OF 1-22 FROM N.A.
TISSUE=Blood
EFERENCE 9 (residues 1 to 363)
AUTHORS Warnecke,C.H., Holzmeister,J., Regitz-Zagrosek,V. and Fleck,E.
TITLE Direct Submission
JOURNAL Submitted (~JUN-1995)
REMARK SEQUENCE OF 1-16 FROM N.A.
TISSUE=Uterus

[FUNCTION] Receptor for angiotensin II. May have a role in cell morphogenesis and related events in growth and development.
[SUBCELLULAR LOCATION] Integral membrane protein.
[TISSUE SPECIFICITY] In adult, highly expressed in myometrium with lower levels in adrenal gland and fallopian tube. Very highly expressed in fetal kidney and intestine.
[SIMILARITY] Belongs to family 1 of G-protein coupled receptors.

EATURES	Location/Qualifiers
source	1..363
	/organism="Homo sapiens"
	/db_xref="taxon:9606"
gene	1..363
	/gene="AGTR2"
Protein	1..363

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                /product="Type-2 angiotensin II receptor"
Region          1..45
                /gene="AGTR2"
                /region_name="Domain"
                /note="Extracellular (Potential)."
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                /note="N-linked (GlcNAc...) (Potential)."
Site            13
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
Site            24
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
Site            29
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
Site            34
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
Region          46..71
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="1 (Potential)."
Region          72..80
                /gene="AGTR2"
                /region_name="Domain"
                /note="Cytoplasmic (Potential)."
Region          81..102
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="2 (Potential)."
Region          103..119
                /gene="AGTR2"
                /region_name="Domain"
                /note="Extracellular (Potential)."
Region          120..140
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="3 (Potential)."
Region          141..160
                /gene="AGTR2"
                /region_name="Domain"
                /note="Cytoplasmic (Potential)."
Region          161..179
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="4 (Potential)."
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                /gene="AGTR2"
                /region_name="Domain"
                /note="Extracellular (Potential)."
Region          209..234
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                /region_name="Transmembrane region"
                /note="5 (Potential)."
Region          235..256
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                /note="Cytoplasmic (Potential)."
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                /region_name="Transmembrane region"
                /note="6 (Potential)."
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                /region_name="Conflict"
                /note="W -> C (in Ref. 5)."
     Region     272
                /gene="AGTR2"
                /region_name="Conflict"
                /note="F -> L (in Ref. 4)."
     Region     279..285
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                /region_name="Domain"
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     Region     286..313
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     Region     314..363
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                /region_name="Domain"
                /note="Cytoplasmic (Potential)."
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                /region_name="Conflict"
                /note="N -> G (in Ref. 4)."
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     121 gsfltlnmfa siffitcmsv dryqsviypf lsqrrnpwqa syivplvwcm aclsslptfy
     181 frdvrtieyl gvnacimafp pekyaqwsag ialmknilgf iiplifiatc yfgirkhllk
     241 tnsygknrit rdqvlkmaaa vvlafiicwl pfhvltflda lawmgvinsc eviavidlal
     301 pfaillgftn scvnpflycf vgnrfqqklr svfrvpitwl qgkresmscr kssslremet
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1: P35374. Type-2 angiotensi...[gi:543779]

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LOCUS       P35374                    363 aa            linear   ROD 15-JUL-1998
DEFINITION  TYPE-2 ANGIOTENSIN II RECEPTOR (AT2).
ACCESSION   P35374
VERSION     P35374  GI:543779
DBSOURCE    swissprot: locus AG22_MOUSE, accession P35374;
            class: standard.
            created: Jun 1, 1994.
            sequence updated: Jun 1, 1994.
            annotation updated: Jul 15, 1998.
            xrefs: gi: 455900, gi: 455901, gi: 439862, gi: 439863, gi: 443584,
            gi: 474274, gi: 487848, , gi: 607834, , gi: 543186
            xrefs (non-sequence databases): GCRDBGCR_0890, GCRDBGCR_1007,
            GCRDBGCR_1010, MGI87966, PROSITEPS00237
KEYWORDS    G-PROTEIN COUPLED RECEPTOR; TRANSMEMBRANE; GLYCOPROTEIN;
            PHOSPHORYLATION.
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 363)
  AUTHORS   Nakajima,M., Mukoyama,M., Pratt,R.E., Horiuchi,M. and Dzau,V.J.
  TITLE     Cloning of cDNA and analysis of the gene for mouse angiotensin II
            type 2 receptor
  JOURNAL   Biochem. Biophys. Res. Commun. 197 (2), 393-399 (1993)
  MEDLINE   94092107
  REMARK    SEQUENCE FROM N.A.
            STRAIN=BALB/C; TISSUE=FETAL
REFERENCE   2  (residues 1 to 363)
  AUTHORS   Ichiki,T., Herold,C.L., Kambayashi,Y., Bardhan,S. and Inagami,T.
  TITLE     Cloning of the cDNA and the genomic DNA of the mouse angiotensin II
            type 2 receptor
  JOURNAL   Biochim. Biophys. Acta 1189 (2), 247-250 (1994)
  MEDLINE   94122216
  REMARK    SEQUENCE FROM N.A.
            STRAIN=BALB/C
REFERENCE   3  (residues 1 to 363)
  AUTHORS   Nahmias,C., Cazaubon,S.M., Sutren,M., Masson,M., Lazard,D.,
            Villageois,P., Elbaz,N. and Strosberg,A.D.
  TITLE     Molecular and functional characterization of angiotensin II AT2
            receptor in neuroblastoma N1E-115 cells
  JOURNAL   Adv. Exp. Med. Biol. 396, 167-173 (1996)
  MEDLINE   96337434
  REMARK    SEQUENCE FROM N.A.
REFERENCE   4  (residues 1 to 363)
  AUTHORS   Horiuchi,M., Koike,G., Yamada,T., Mukoyama,M., Nakajima,M. and
            Dzau,V.J.
  TITLE     The growth-dependent expression of angiotensin II type 2 receptor
            is regulated by transcription factors interferon regulatory
            factor-1 and -2
  JOURNAL   J. Biol. Chem. 270 (34), 20225-20230 (1995)
  MEDLINE   95378283
  REMARK    SEQUENCE FROM N.A.
            STRAIN=BALB/C; TISSUE=LIVER
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Annexe III

```
)MMENT      [FUNCTION] RECEPTOR FOR ANGIOTENSIN II. MAY HAVE A ROLE IN CELL
            MORPHOGENESIS AND RELATED EVENTS IN GROWTH AND DEVELOPMENT.
            [SUBCELLULAR LOCATION] INTEGRAL MEMBRANE PROTEIN.
            [TISSUE SPECIFICITY] ABUNDANT IN FETUS, LOWER LEVELS IN ADULT
            BRAIN.
            [PTM] CARBOXYL-TERMINAL SER OR THR RESIDUES MAY BE PHOSPHORYLATED.
            [SIMILARITY] BELONGS TO FAMILY 1 OF G-PROTEIN COUPLED RECEPTORS.
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                        /db_xref="taxon:10090"
     gene               1..363
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     Protein            1..363
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                        /product="TYPE-2 ANGIOTENSIN II RECEPTOR"
     Region             1..45
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                        /region_name="Domain"
                        /note="EXTRACELLULAR."
     Site               4
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     Site               13
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                        /site_type="glycosylation"
     Site               24
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     Site               29
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                        /site_type="glycosylation"
     Site               34
                        /gene="AGTR2"
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                        /region_name="Transmembrane region"
                        /note="1."
     Region             72..80
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                        /note="CYTOPLASMIC."
     Region             81..102
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                        /region_name="Transmembrane region"
                        /note="2."
     Region             103..119
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                        /region_name="Domain"
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     Region             120..140
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                        /region_name="Domain"
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     Region             161..179
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Annexe III

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                     /note="EXTRACELLULAR."
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     Region          235..256
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                     /region_name="Domain"
                     /note="CYTOPLASMIC."
     Region          257..278
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     Region          279..285
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     Region          286..313
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     Region          314..363
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                     /site_type="phosphorylation"
                     /note="(BY PKC)."
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       61 vnivvvslfc cqkgpkkvss iyifnlalad llllatlplw atyysyrydw lfgpvmckvf
      121 gsfltlnmfa siffitcmsv dryqsviypf lsqrrnpwqa syvvplvwcm aclsslptfy
      181 frdvrtieyl gvnacimafp pekyaqwsag ialmknilgf iiplifiatc yfgirkhllk
      241 tnsygknrit rdqvlkmaaa vvlafiicwl pfhvltflda ltwmgiinsc eviavidlal
      301 pfaillgftn scvnpflycf vgnrfqqklr svfrvpitwl qgkretmscr kgsslremdt
      361 fvs
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Souris Mutagenèse

Annexe III

NCBI

Sequence Revision History

Revision history for P35374

GI	Version	Update Date	Status	I	II
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543779	n/a	Jan 20 2004 9:10 AM	Dead	○	◉
543779	n/a	Dec 16 2003 8:40 AM	Dead	○	○
543779	n/a	Nov 11 2003 8:21 AM	Dead	○	○
543779	n/a	Sep 17 1999 1:22 PM	Dead	○	○
543779	n/a	Jul 21 1998 4:10 AM	Dead	○	○
543779	n/a	Apr 10 1998 12:54 AM	Dead	○	○
543779	n/a	Feb 7 1998 3:51 AM	Dead	○	○
543779	n/a	Feb 2 1998 2:26 PM	Dead	○	○
543779	n/a	Oct 9 1997 9:39 PM	Dead	○	○
543779	n/a	Dec 3 1996 9:33 PM	Dead	○	○
543779	n/a	Jun 3 1996 4:48 PM	Dead	○	○
543779	n/a	Jan 28 1996 6:15 PM	Dead	○	○
543779	n/a	Dec 1 1994 3:42 PM	Dead	○	○
543779	n/a	Sep 22 1994 4:36 PM	Dead	○	○

Accession P35374 was first seen at NCBI on Sep 22 1994 4:36 PM

1: P35374. Type-2 angiotensi...[gi:543779]

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LOCUS       P35374                    363 aa            linear   ROD 15-MAR-2004
DEFINITION  Type-2 angiotensin II receptor (AT2).
ACCESSION   P35374
VERSION     P35374  GI:543779
SOURCE      swissprot: locus AG22_MOUSE, accession P35374;
            class: standard.
            created: Jun 1, 1994.
            sequence updated: Jun 1, 1994.
            annotation updated: Mar 15, 2004.
            xrefs: gi: 455900, gi: 455901, gi: 439862, gi: 439863, gi: 443584,
            gi: 474274, gi: 487848, gi: 1881537, gi: 607834, gi: 607836, gi:
            13277866, gi: 13277867, gi: 2137148
            xrefs (non-sequence databases): HSSPP34996, MGI87966,
            InterProIPR000276, PfamPF00001, PRINTSPR00237, PROSITEPS00237,
            PROSITEPS50262
KEYWORDS    G-protein coupled receptor; Transmembrane; Glycoprotein;
            Phosphorylation.
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 363)
  AUTHORS   Nakajima,M., Mukoyama,M., Pratt,R.E., Horiuchi,M. and Dzau,V.J.
  TITLE     Cloning of cDNA and analysis of the gene for mouse angiotensin II
            type 2 receptor
  JOURNAL   Biochem. Biophys. Res. Commun. 197 (2), 393-399 (1993)
  MEDLINE   94092107
   PUBMED   8267573
  REMARK    SEQUENCE FROM N.A.
            STRAIN=BALB/c; TISSUE=Fetal
REFERENCE   2  (residues 1 to 363)
  AUTHORS   Ichiki,T., Herold,C.L., Kambayashi,Y., Bardhan,S. and Inagami,T.
  TITLE     Cloning of the cDNA and the genomic DNA of the mouse angiotensin II
            type 2 receptor
  JOURNAL   Biochim. Biophys. Acta 1189 (2), 247-250 (1994)
  MEDLINE   94122216
   PUBMED   8292631
  REMARK    SEQUENCE FROM N.A.
            STRAIN=BALB/c
REFERENCE   3  (residues 1 to 363)
  AUTHORS   Nahmias,C., Cazaubon,S.M., Sutren,M., Masson,M., Lazard,D.,
            Villageois,P., Elbaz,N. and Strosberg,A.D.
  TITLE     Molecular and functional characterization of angiotensin II AT2
            receptor in neuroblastoma N1E-115 cells
  JOURNAL   Adv. Exp. Med. Biol. 396, 167-173 (1996)
  MEDLINE   96337434
   PUBMED   8726696
  REMARK    SEQUENCE FROM N.A.
REFERENCE   4  (residues 1 to 363)
  AUTHORS   Horiuchi,M., Koike,G., Yamada,T., Mukoyama,M., Nakajima,M. and
            Dzau,V.J.
  TITLE     The growth-dependent expression of angiotensin II type 2 receptor
            is regulated by transcription factors interferon regulatory
```

Acwsc

TTT

factor-1 and -2
JOURNAL J. Biol. Chem. 270 (34), 20225-20230 (1995)
MEDLINE 95378283
PUBMED 7650042
REMARK SEQUENCE FROM N.A.
STRAIN=BALB/c; TISSUE=Liver
EFERENCE 5 (residues 1 to 363)
AUTHORS Strausberg,R.L., Feingold,E.A., Grouse,L.H., Derge,J.G., Klausner,R.D., Collins,F.S., Wagner,L., Shenmen,C.M., Schuler,G.D., Altschul,S.F., Zeeberg,B., Buetow,K.H., Schaefer,C.F., Bhat,N.K., Hopkins,R.F., Jordan,H., Moore,T., Max,S.I., Wang,J., Hsieh,F., Diatchenko,L., Marusina,K., Farmer,A.A., Rubin,G.M., Hong,L., Stapleton,M., Soares,M.B., Bonaldo,M.F., Casavant,T.L., Scheetz,T.E., Brownstein,M.J., Usdin,T.B., Toshiyuki,S., Carninci,P., Prange,C., Raha,S.S., Loquellano,N.A., Peters,G.J., Abramson,R.D., Mullahy,S.J., Bosak,S.A., McEwan,P.J., McKernan,K.J., Malek,J.A., Gunaratne,P.H., Richards,S., Worley,K.C., Hale,S., Garcia,A.M., Gay,L.J., Hulyk,S.W., Villalon,D.K., Muzny,D.M., Sodergren,E.J., Lu,X., Gibbs,R.A., Fahey,J., Helton,E., Ketteman,M., Madan,A., Rodrigues,S., Sanchez,A., Whiting,M., Madan,A., Young,A.C., Shevchenko,Y., Bouffard,G.G., Blakesley,R.W., Touchman,J.W., Green,E.D., Dickson,M.C., Rodriguez,A.C., Grimwood,J., Schmutz,J., Myers,R.M., Butterfield,Y.S.N., Krzywinski,M.I., Skalska,U., Smailus,D.E., Schnerch,A., Schein,J.E., Jones,S.J.M. and Marra,M.A.
TITLE Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 99 (26), 16899-16903 (2002)
MEDLINE 22388257
PUBMED 12477932
REMARK SEQUENCE FROM N.A.
STRAIN=C57BL/6J; TISSUE=Mammary gland
OMMENT ---
This SWISS-PROT entry is copyright. It is produced through a collaboration between the Swiss Institute of Bioinformatics and the EMBL outstation - the European Bioinformatics Institute. The original entry is available from http://www.expasy.ch/sprot and http://www.ebi.ac.uk/sprot
---.
[FUNCTION] Receptor for angiotensin II. May have a role in cell morphogenesis and related events in growth and development.
[SUBCELLULAR LOCATION] Integral membrane protein.
[TISSUE SPECIFICITY] Abundant in fetus, lower levels in adult brain.
[PTM] Carboxyl-terminal Ser or Thr residues may be phosphorylated.
[SIMILARITY] Belongs to family 1 of G-protein coupled receptors.

EATURES	Location/Qualifiers
source	1..363
	/organism="Mus musculus"
	/db_xref="taxon:10090"
gene	1..363
	/gene="AGTR2"
Protein	1..363
	/gene="AGTR2"
	/product="Type-2 angiotensin II receptor"
Region	1..45
	/gene="AGTR2"
	/region_name="Domain"
	/note="Extracellular (Potential)."
Site	4
	/gene="AGTR2"
	/site_type="glycosylation"
	/note="N-linked (GlcNAc...) (Potential)."
Site	13

Annexe III

```
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                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
     Site       24
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
     Site       29
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
     Site       34
                /gene="AGTR2"
                /site_type="glycosylation"
                /note="N-linked (GlcNAc...) (Potential)."
     Region     46..71
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="1 (Potential)."
     Region     72..80
                /gene="AGTR2"
                /region_name="Domain"
                /note="Cytoplasmic (Potential)."
     Region     81..102
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="2 (Potential)."
     Region     103..119
                /gene="AGTR2"
                /region_name="Domain"
                /note="Extracellular (Potential)."
     Region     120..140
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="3 (Potential)."
     Region     141..160
                /gene="AGTR2"
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                /note="4 (Potential)."
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                /region_name="Domain"
                /note="Extracellular (Potential)."
     Region     209..234
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="5 (Potential)."
     Region     235..256
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                /region_name="Domain"
                /note="Cytoplasmic (Potential)."
     Region     257..278
                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="6 (Potential)."
     Region     279..285
                /gene="AGTR2"
                /region_name="Domain"
                /note="Extracellular (Potential)."
     Region     286..313
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Annexe III

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                /gene="AGTR2"
                /region_name="Transmembrane region"
                /note="7 (Potential)."
     Region     314..363
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                /region_name="Domain"
                /note="Cytoplasmic (Potential)."
     Site       354
                /gene="AGTR2"
                /site_type="phosphorylation"
                /note="(BY PKC) (POTENTIAL)."
RIGIN
       1 mkdnfsfaat srnitssrpf dnlnatgtne safncshkps dkhleaipvl yymifvigfa
      61 vnivvvslfc cqkgpkkvss iyifnlalad llllatlplw atyysyrydw lfgpvmckvf
     121 gsfltlnmfa siffitcmsv dryqsviypf lsqrrnpwqa syvvplvwcm aclsslptfy
     181 frdvrtieyl gvnacimafp pekyaqwsag ialmknilgf iiplifiatc yfgirkhllk
     241 tnsygknrit rdqvlkmaaa vvlafiicwl pfhvltflda ltwmgiinsc eviavidlal
     301 pfaillgftn scvnpflycf vgnrfqqklr svfrvpitwl qgkretmscr kgsslremdt
     361 fvs
```

Nucleotide (3/09)

1: BE652532. UI-M-AM0-adp-b-06...[gi:9978333] Links

IDENTIFIERS

dbEST Id: **5909367**
EST name: UI-M-AM0-adp-b-06-0-UI.r1
GenBank Acc: BE652532
GenBank gi: 9978333

CLONE INFO
Clone Id: UI-M-AM0-adp-b-06-0-UI (5')
Source: The NIH - University of Iowa Brain Molecular Anatomy Project: NIH-Iowa BMAP (Bento Soares, Thomas Casavar Val Sheffield)
Id as DNA: UI-M-AM0-adp-b-06-0-UI
Id in host: UI-M-AM0-adp-b-06.r1
DNA type: cDNA

PRIMERS
Sequencing: M13 Reverse
PolyA Tail: Unknown

SEQUENCE

GCTGCTTCAAGCGCCTGTGAGAAGCTAGAAAAGGCTAGGACTGACTTACAGA
CAAGAATTTGTCCAGAAACTAAACCAGCAGCATCAGACAGACCGGACGGAAC
CGGCTGAAGGACTTATACACCGCAGAGTGTGAGAAGCTTCAGAGCATTTACA
GCAGAAAAATATAAAACTCAACTGCAAGAGCAGTTTGACAACTTAAACGCCC
ACCACTAAGCTTGAGATTGAAGCTAGCCACTCGGAGAAGGTGGAATTGCTGA
TATGAAACCTCCCTTTCAGAAATCAAGAAGAGCCATGAGATGGAGAAGAAGT
GATCTGCTTAATGAGAAGCAGGAATCGCTGGAGAAACAAATCAATGATCTGA
AACGATGCTTTAAACGAAAGGTTGAAATCAGAGGAGCAAAAGCAACTGTCAA
GCGAATTCAAAAAACCCTCAGGTCATGTATCTGGAGC

Entry Created: Sep 6 2000
Last Updated: Sep 6 2000

COMMENTS

cDNA Library Preparation: M.B. Soares Lab Clone distribution: Researchers may obtain BMAP cDNA clone RESEARCH GENETICS. It should be noted that Bento Soa generating a small number of additional specialized non-redundant arrays of BMAP cDNAs whose availabilit be considered under appropriate and limited collabor arrangements

LIBRARY
Lib Name: NIH_BMAP_MAM

Strain:	C57BL/6J
Develop. stage:	27-32 days
Lab host:	DH10B (Life Technologies)
Vector:	pT7T3D-Pac (Pharmacia) with a modified polylinker
R. Site 1:	Not I
R. Site 2:	Eco RI
Description:	The NIH_BMAP_MAM library is a non-normalized library constructed from mouse amygdala. The tag is a string nucleotides present between the Not I site and the c track. The library was constructed as described by E Lennon and Soares, Genome Research 6: 791-806, 1996. provided by Ms. Annie Novakovich, Zivic-Miller Labor

SUBMITTER

Name:	Chin, H
Institution:	National Institute of Mental Health
Address:	6001 Executive Blvd. Room 7N-7190, MSC 9643, Bethesc 20892-9643, USA
Tel:	301 443 1706
Fax:	301 443 9890
E-mail:	mEST@mail.nih.gov

CITATIONS

Medline UID:	97044477
Title:	Normalization and subtraction: two approaches to fac gene discovery
Authors:	Bonaldo,M.F., Lennon,G., Soares,M.B.
Citation:	Genome Res. 6 (9): 791-806 1996

MAP DATA

Revision history for BE652532

GI	Version	Update Date	Status
9978333	1	Sep 6 2000 6:24 PM	Live

Accession BE652532 was first seen at NCBI on Sep 6 2000 6:24 PM

Entrez

Search for Genes
LocusLink provides curated information for human, fruit fly, mouse, rat, and zebrafish

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Clusters of orthologous groups

Protein reviews on the web

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ANNEX IVb

1: Genome Res. 1996 Sep;6(9):791-806.

Normalization and subtraction: two approaches to facilitate gene discovery.

Bonaldo MF, Lennon G, Soares MB.

Department of Psychiatry, College of Physicians and Surgeons of Columbia University, New York, New York, USA.

Large-scale sequencing of cDNAs randomly picked from libraries has proven to be a very powerful approach to discover (putatively) expressed sequences that, in turn, once mapped, may greatly expedite the process involved in the identification and cloning of human disease genes. However, the integrity of the data and the pace at which novel sequences can be identified depends to a great extent on the cDNA libraries that are used. Because altogether, in a typical cell, the mRNAs of the prevalent and intermediate frequency classes comprise as much as 50-65% of the total mRNA mass, but represent no more than 1000-2000 different mRNAs, redundant identification of mRNAs of these two frequency classes is destined to become overwhelming relatively early in any such random gene discovery programs, thus seriously compromising their cost-effectiveness. With the goal of facilitating such efforts, previously we developed a method to construct directionally cloned normalized cDNA libraries and applied it to generate infant brain (INIB) and fetal liver/spleen (INFLS) libraries, from which a total of 45,192 and 86,088 expressed sequence tags, respectively, have been derived. While improving the representation of the longest cDNAs in our libraries, we developed three additional methods to normalize cDNA libraries and generated over 35 libraries, most of which have been contributed to our integrated Molecular Analysis of Genomes and Their Expression (IMAGE) Consortium and thus distributed widely and used for sequencing and mapping. In an attempt to facilitate the process of gene discovery further, we have also developed a subtractive hybridization approach designed specifically to eliminate (or reduce significantly the representation of) large pools of arrayed and (mostly) sequenced clones from normalized libraries yet to be (or just partly) surveyed. Here we present a detailed description and a comparative analysis of four methods that we developed and used to generate normalize cDNA libraries from human (15), mouse (3), rat (2), as well as the parasite Schistosoma mansoni (1). In addition, we describe the construction and preliminary characterization of a subtracted liver/spleen library (INFLS-SI) that resulted from the elimination (or reduction of representation) of -5000 INFLS-IMAGE clones from the INFLS library.

PMID: 8889548 [PubMed - indexed for MEDLINE]

IDENTIFIERS

dbEST Id:	**1607105**
EST name:	vx39f05.r1
GenBank Acc:	AA880300
GenBank gi:	2989283

CLONE INFO

Clone Id:	IMAGE:1277601 (5')
Source:	IMAGE Consortium, LLNL
DNA type:	cDNA

PRIMERS

Sequencing:	-28m13 rev1 ET from Amersham
PolyA Tail:	Unknown

SEQUENCE

CTTTCCCGTGGTAACACCAAGTTTGAAGCGCTGACAGTTGTGATCCAGCACC
GAGCGGGAGGAAGCACTGAAGCAACACAAAACCCTCTCTCAAGAACTTGTCA
GGAGAGCTAGTTGCTGCTTCAAGCGCCTGTGAGAAGCTAGAAAAGGCTAGGC
CAGACAGCGTATCAAGAATTTGTCCAGAAACTAAACCAGCAGCATCAGACAC
GAACTGGAGAACCGGCTGAAGGACTTATACACCGCAGAGTGTGAGAAGCTTC
TACATTGAGGAGGCAGAAAAATATAAAACTCAACTGCAAGAGCAGTTTGACA
GCCGCCCATGAGACCACTAAGCTTGAGATTGAAGCTAGCCACTCGGAGAAGC
CTGAAGAAGACCTATGAAACCTCCCTTTCAGAAATCAAGAAGAGCCATGAGA
AAGTCACTGGAGGATCTGCT

Quality: High quality sequence stops at base: 464

Entry Created:	Mar 26 1998
Last Updated:	Mar 26 1998

COMMENTS

This clone is available royalty-free through LLNL ; the IMAGE Consortium (info@image.llnl.gov) for furth information.
MGI:669401

LIBRARY

Lib Name:	Stratagene mouse lung 937302
Organism:	Mus musculus
Strain:	C57BL/6 x CBA
Sex:	female
Organ:	lung
Tissue type:	lung
Develop. stage:	6-8 month old

Vector:	pBluescript SK-
R. Site 1:	EcoRI
R. Site 2:	XhoI
Description:	Cloned unidirectionally. Primer: Oligo dT. 6-8 montl female lung and 1.5 year old male lung were source c Average insert size: 1.5 kb; Uni-ZAP XR Vector; ~5' sequence: 5' GAATTCGGCACGAG 3' ~3' adaptor sequence: CTCGAGTTTTTTTTTTTTTTT 3'

SUBMITTER

Name:	Marra M/Mouse EST Project
Lab:	WashU-HHMI Mouse EST Project
Institution:	Washington University School of MedicineP
Address:	4444 Forest Park Parkway, Box 8501, St. Louis, MO 6
Tel:	314 286 1800
Fax:	314 286 1810
E-mail:	mouseest@watson.wustl.edu

CITATIONS

Title:	The WashU-HHMI Mouse EST Project
Authors:	Marra,M., Hillier,L., Allen,M., Bowles,M., Dietrich, Dubuque,T., Geisel,S., Kucaba,T., Lacy,M., Le,M., Ma Morris,M., Schellenberg,K., Steptoe,M., Tan,F., Unde , Moore,B., Theising,B., Wylie,T., Lennon,G., Soares Wilson,R., Waterston,R.
Year:	1996
Status:	Unpublished

MAP DATA

Revision history for AA880300

GI	Version	Update Date	Status
2989283	1	Mar 27 1998 12:37 AM	Live

Accession AA880300 was first seen at NCBI on Mar 27 1998 12:37 AM

Entrez

Search for Genes
LocusLink provides curated information for human, fruit fly, mouse, rat, and zebrafish

Help|FAQ

Batch Entrez:
Upload a file of GI or accession numbers to retrieve protein or nucleotide sequences

Check sequence revision history

How to create WWW links to Entrez

LinkOut

Cubby

Related resources

BLAST

Reference sequence project

LocusLink

Clusters of orthologous groups

Protein reviews on the web

Blast 2 Sequences results

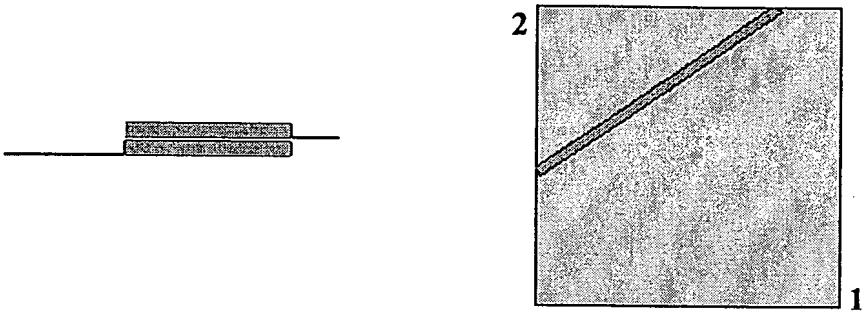
PubMed Entrez BLAST OMIM Taxonomy Structure

LAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.6 [Apr-09-2003]

latch: 1 Mismatch: -2 gap open: 5 gap extension: 2
_dropoff: 50 expect: 10.000 wordsize: 11 Filter ☐ Align

equence lcl|seq_1 **Length** 354 (1 .. 354)

equence gi 2989283 vx39f05.r1 Stratagene mouse lung 937302 Mus musculus cDNA clone IMAGE:1277601 5'. **Length** 500 (1 .. 500)



OTE:The statistics (bitscore and expect value) is calculated based on the size of nr database

OTE:If protein translation is reversed, please repeat the search with reverse strand of the query sequence

```
core =  535 bits (278), Expect = e-149
dentities = 278/278 (100%)
Strand = Plus / Plus
```

```
uery: 1   catcagacagaccggacggaactggagaaccggctgaaggacttatacaccgcagagtgt 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
bjct: 223 catcagacagaccggacggaactggagaaccggctgaaggacttatacaccgcagagtgt 282

uery: 61  gagaagcttcagagcatttacattgaggaggcagaaaaatataaaactcaactgcaagag 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
bjct: 283 gagaagcttcagagcatttacattgaggaggcagaaaaatataaaactcaactgcaagag 342

uery: 121 cagtttgacaacttaaacgccgcccatgagaccactaagcttgagattgaagctagccac 180
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
bjct: 343 cagtttgacaacttaaacgccgcccatgagaccactaagcttgagattgaagctagccac 402

uery: 181 tcggagaaggtggaattgctgaagaagacctatgaaacctccctttcagaaatcaagaag 240
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
bjct: 403 tcggagaaggtggaattgctgaagaagacctatgaaacctccctttcagaaatcaagaag 462

uery: 241 agccatgagatggagaagaagtcactggaggatctgct 278
          |||||||||||||||||||||||||||||||||||||
bjct: 463 agccatgagatggagaagaagtcactggaggatctgct 500
```

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PU time:     0.01 user secs.       0.01 sys. secs          0.02 total secs.

ambda      K      H
   1.33    0.621     1.12

apped
ambda      K      H
   1.33    0.621     1.12


atrix: blastn matrix:1 -2
ap Penalties: Existence: 5, Extension: 2
ımber of Hits to DB: 2
ımber of Sequences: 0
ımber of extensions: 2
ımber of successful extensions: 1
ımber of sequences better than 10.0: 1
ımber of HSP's better than 10.0 without gapping: 1
ımber of HSP's successfully gapped in prelim test: 0
ımber of HSP's that attempted gapping in prelim test: 0
ımber of HSP's gapped (non-prelim): 1
ength of query: 354
ength of database: 10,249,863,584
ffective HSP length: 24
ffective length of query: 330
ffective length of database: 10,249,863,560
ffective search space: 3382454974800
ffective search space used: 3382454974800
: 0
: 0
l: 6 (11.5 bits)
?: 26 (50.0 bits)
l: 12 (23.8 bits)
?: 20 (39.1 bits)
```

CURRICULUM VITAE
ARTHUR DONNY STROSBERG

PERSONAL INFORMATION:
Hybrigenics SA
3-5 Impasse Reille
75014 Paris (France)
Phone: 33 (0) 1 58 10 38 00
Fax: 33 (0) 1 58 10 38 40
Email: adstrosberg@hybrigenics.fr
Web site: http://www.hybrigenics.com

Address (home)	66, rue de Javel 75015 Paris (France) Telephone: +33-1 45 79 52 42 Telefax: +33-1 45 77 76 98
Born:	March 2, 1945 at Montreux (Switzerland)
Citizenship:	Belgian
Marital Status	Married, two children

EDUCATION

1966	License in Chemistry Free University of Brussels (Belgium)
1970	Doctorate in Sciences (Chemistry) Free University of Brussels (Belgium)

PROFESSIONAL EXPERIENCE

1969-1972	Instructor in Protein Chemistry Free University of Brussels (Belgium)
1970-1973	Research Fellow in Medicine Massachusetts General Hospital, Boston (USA)
1972-1973	Instructor in Medicine, Harvard Medical School, Boston (USA)
1972-1977	Assistant Professor in Protein Chemistry Free University of Brussels (Belgium)
1974-1980	Professor Biochemical Pathology Free University of Brussels (Belgium)
1977-1990	Chair Professor Biochemistry and Immunology University of Paris VII (France)

1982-1990	Chairman, Immunology Graduate School Ile de France
1982-1985	Chairman, Department of Biochemistry, University Paris VII (France)
1986-1990	Chief, Unit of Molecular Biology of Receptors (Institut Pasteur, Paris)
1986 -1990	Chairman of the Commission des Spécialistes, Section 37, University Paris VII, Conseil National des Universités.
1990 -1998	Director Unit of Molecular Immuno-Pharmacology, CNRS and Vice-President Institut Cochin de Génétique Moléculaire (Paris)
1993	Visiting Professor, Harvard Medical School (Boston, USA)
1998 -	Founder and Vice-President France Biotech (Communication and relation with research organisations)
1999 -	Founder, Chairman, President & CEO Hybrigenics

HONORS AND AWARDS:

1966-1968	I.R.S.I.A. - I.W.O.N.L. Fellowship
1970-1973	Fullbright Honorary Fellow
1970-1973 (EMBO)	Long-term Research Fellow European Molecular Organization
1974:	International Prize for Medical Research
1988	Knight in the Order of Merit
1992	Vermeil Medal of the Society for Encouragement of Progress
1992	Boehringer Ingelheim Lecturer
2001	Technology Pioneer World Economic Forum (Davos)

MEMBERSHIPS:

1969 -	Belgische Vereniging voor Biochemie (Belgie)
1972 -	New York Academy of Sciences
1973 -	American Association of Immunologists

1974 -	Nederlandse Vereniging voor Immunologie
1974 -	Société Française d'Immunologie
1975 -	The Biochemical Society
1976 - 1980	Inter-University Protein Sequence Group GISP-IGES (Founder and Chairman)
1978 - 1980	Belgian Society for Immunology (Founder and Chairman)
1979 - 1982	Associate Editor *Molecular Immunology (Immunochemistry)*
1983 -	European Molecular Biology Organization (EMBO)
1984 - 1989	Associate Editor *Molecular and Cellular Endocrinology*
1987 - 1995	Committee of French Drug Agency for approval of Biotechnology based drugs, Health Ministry
1988 - 1990	Associate Editor *Journal of Autoimmunity*
1990 -	Associate Editor *Current Opinion in Biotechnology*
1991 - 1994	Associate Editor *Vaccine Research*
1991 -	Associate Editor *Biologicals*
1991 - 1999	Associate Member of the Academy of Sciences Committee for the Applications of Sciences (C.A.D.A.S.)
1991 - 1994	Chairman, Scientific Committee of the Ministry of Research and Technology for Gene Therapy-based projects
1992 - 1994	European Editor of "*Receptors and Channels*"
1994 - 1997	Associate Editor *Immunological Investigations*
2003 -	Associate Editor *Clinical Proteomics*
2003 -	Associate Editor *Targets*
2003 -	Associate Editor *Genetic Engineering News*

COMMITTEE MEMBERSHIPS:

a. *Biotechnology Incubators (past and current)*

Paris BioParc (Business Incubator)
Genopole Entreprises
Paris Biotech (Cochin Institute)
BioTop (Pasteur Institute)
Biodiscovery (Rothschild group)

b. *General Interest*

CODEV (Committee for Paris Economic Development)
Government Fund of Funds (FPCR)
SITEF

MAJOR SCIENTIFIC ACHIEVEMENTS

- One of two laboratories to produce and sequence **antibodies of restricted heterogeneity** against microbial antigens pneumoccus, micrococus (*Biochemistry*, 11, 4978-4985, 1972).
- First to produce **monoclonal antibodies** in 1973, by immortalizing, using SV40 rabbit lymphocytes, producing anti-pneumoccal antibodies (*Proc. Natl. Acad. Sci. USA* 71, 263-264, 1974).
- First to reveal the existence and to characterize **"latent allotypes"** in rabbits (*J. Immunol.* 113, 1313-1318, 1974; *Immunogenetics*, 4, 499-513, 1977).
- First to **isolate, to separate from adenylyl cyclase and to characterize a G protein-coupled receptor:** the β-adrenergic receptor from turkey erythrocytes (*Proc. Natl. Acad. Sci. USA* 74, 3710-3714, 1977).
- First to demonstrate that **anti-idiotypic antibodies** raised against anti-catecholamine antibodies, **can recognize the β-adrenergic receptor** (*J. Exp. Med.* 157, 1369-1378, 1983).
- One of two laboratories to first **clone, sequence and express the human β-adrenergic receptor** (*Proc. Natl. Acad. Sci. USA* 84, 6995-6999, 1987).
- First to achieve **functional expression of G protein-coupled receptors in *E. coli*** bacteria: β-adrenergic (*Proc. Natl. Acad. Sci. USA* 85, 7551-7555, [1988]; *EMBO J.* 9, 1471-1476, [1990]), 5HT1A serotonine (*J. Biol. Chem.* 267, 8200-8206, 1992) and β-adrenergic receptor-$G_{s\alpha}$ fusion protein (*Proc. Natl. Acad. Sci. USA* 91, 8827-8831, 1994).
- **First to clone, sequence and express the human β-adrenergic receptor** (*Science* 245, 1118-1121, 1989), to describe its pharmacology (*Mol. Pharmacol.* 44, 1094-1104, 1993), its expression in human (*J. Clin. Invest.* 91, 344-349, 1993).
- One of two laboratories to first **clone, sequence and express the human AT2 angiotensin 1 receptor** (*Eur. J. Biochem.* 220, 919-926,1994) and to demonstrate coupling of its murine counterpart to a **tyrosine phosphatase** (*Receptors & Channels* 2, 271-280, 1994).
- One of the two laboratories to first **identify a natural variant of human β-adrenergic receptor** (*New Engl. J. Med.* 333, 343-347, 1995) and to **correlate this single substitution with increased obesity** (*New Engl. J. Med.* 333, 352-354, 1995).

- **Peer-reviewed publications:** 364
- **Reviews and chapters in books:** 75
- **Books:** 5

MAJOR INDUSTRY-RELATED ACHIEVEMENTS

- 21 issued patents in USA, Europe and other countries. Several licensed to major pharmaceutical companies.

- Major multiannual research and development contracts with international pharmaceutical companies including, Abbott, Bristol-Myers-Squibb, Ciba-Geigy, Hoffmann-LaRoche, Janssen Pharmaceutica, Servier. Sumitomo Chemicals.

- Multiannual personal consultancy agreements with, among others: Abbott, Genentech, Bristol-Myers-Squibb.

- Founder of several biotechnology companies including Chemunex SA (1984), Ideon/Incyte Inc. (1987/1993), Vetigen SARL (1992), Pharmaceutical Peptides Inc./Praecis (1994), Neurotech SA (1995), Hybrigenics (1998).

- Chairman, President and CEO Hybrigenics

Prof. A. Donny STROSBERG

publications 1969-2003

1. Strosberg, A.D. and Kanarek, L. "Immunochemical studies on hen's egg white lysozyme. Effect of formylation of the tryptophan residues." FEBS Letters, 5, 324-326 (1969).

2. Strosberg, A.D. and Kanarek, L. "Ratio of the Fab fragments I and II from goat antibodies and normal gamma globulins." FEBS Letters 6, 28-30 (1970).

3. Strosberg, A.D. and Kanarek, L. "Immunochemical studies on hen's egg white lysozyme. The role of the lysine, the histidine, and the methionine residues." Eur. J. Biochem. 14, 161-168 (1970).

4. Strosberg, A.D., Nihoul-De Coninck, C. and Kanarek, L. "Weak immunological cross-reaction between bovine α-lactalbumine and hen's egg-white lysozyme." Nature 227, 1241-1341 (1970).

5. Strosberg, A.D., Van Hoeck, B. and Kanarek, L. "Immunochemical studies on hen's egg-white lysozyme. Effect of selective nitration of the three tyrosine residues." Eur. J. Biochem. 19, 36-41 (1971).

6. Holowka, D.A., Strosberg, A.D., Kimball, J.W., Haber, E. and Cathou, R.E. "Changes of intrinsic circular dichroism of several homogeneous anti-type III-pneumococcal antibodies on binding of a small hapten." Proc. Natl. Acad. Sci. USA 69, 3399-3403 (1972).

7. Strosberg, A.D., Fraser, K.J., Margolies, M.N. and Haber, E. "Amino acid sequence of rabbit pneumococcal antibody. I. Light chain cystein containing peptides." Biochemistry 11, 4978-4985 (1972).

8. Chen, F.W., Strosberg, A.D. and Haber, E. "Evolution of the immune response to type III and VII pneumococcal polysaccharides." J. Immunol. 110, 98-106 (1973).

9. Jaton, J.C., Braun, D.G., Strosberg, A.D., Haber, E. and Morris, J.E. "Restricted rabbit antibodies: amino acid sequences of rabbit H chains of allotype a1, a2 and a3 in the region 80 to 94." J. Immunol. 111, 1838-1843 (1973).

10. Collins, J.J., Black, P.H., Strosberg, A.D., Haber, E., and Bloch, K.J. "Transformation by simian virus 40 of spleen cells from a hyperimmune rabbit: evidence for synthesis of immunoglobulin by the transformed cells." Proc. Natl. Acad. Sci. USA, 71, 260-262 (1974).

11. Strosberg, A.D., Collins, J.J., Black, P.H., Malamud, D., Wilbert, S., Bloch, K.J., and Haber, E. "Transformation by simian virus 40 of spleen cells from a hyperimmune rabbit: demonstration of production of specific antibody to the immunizing antigen." Proc. Natl. Acad. Sci. USA 71, 263-264 (1974).

12. Strosberg, A.D., Jeffery, R.A., Freier, L.J., and Connolly, W.J. "Accelerated liquid isoelectric focusing using a direct current source that maintains constant power." Analyt. Biochem. 69, 76-83 (1975).

13. Smith, T.W., Wagner, H. Jr., Strosberg, A.D., and Young, M. "Solubilization and partial purification of canine myocardial (Na^+ $^+K^+$) ATPase." Ann. N.Y. Acad. Sci. 242, 53-68 (1974).

14. Strosberg, A.D., Hamers-Casterman, C., Van der Loo, W. and Hamers, R. "A rabbit with the allotypic phenotype a1a2a3 b4b5b6." J. Immunol. 113, 1313-1318 (1974).

15. Bollengier, F., Strosberg, A.D., Karcher, D., Lowenthal, A. and Rabinovitch-Mahler, A. "Immunological study of the agent responsible for subacute sclerosing panencephalitis and biochemical characterization of measles antibody in subacute sclerosing panencephalitis." Med. Microbiol. Immunol. 160, 173-177 (1974).

16. Vauquelin, G., Lacombe, M.L., Hanoune, J. and Strosberg, A.D. "Stability of isoproterenol bound to cyanogen-activated agarose." Biochem. Biophys. Res. Comm. 64, 1076-1082 (1975).

17. Hamers, R., Hamers-Casterman, C., Van der Loo, W., De Baetselier, P., and Strosberg, A.D. "Rheumatoid factor appearance in *Micrococcus lysodeikticus* immunization and its interference with allotype specific reactions." Z. Immunitat Forschung 149, 187-192 (1975).

18. Margolies, M.N., Cannon, E.L., Strosberg, A.D. and Haber, E. "Diversity of light chain variable region sequences among rabbit antibodies elicited by the same antigens." Proc. Natl. Acad. Sci. USA 72, 2180-2184 (1975).

19. Strosberg, A.D., Karcher, D., and Lowenthal, A. "Structural homogeneity of human subacute sclerosing panencephalitis antibodies." J. Immunol. 115, 157-160 (1975).

20. Mareschal, J.C., Schonne, E., Crichton, R.R., and Strosberg, A.D. "Amino acid sequence homology in the active site of rabbit, beef, whale and calamary muscle aldolase." FEBS Letters 54, 97-99 (1975).

21. Strosberg, A.D., Margolies, M.N., and Haber, E. "The interdomain disulfide bond of a homogeneous rabbit pneumococcal antibody light chain." J. Immunol. 115, 1422-1424 (1975).

22. Strosberg, A.D. "A possible control by regulatory allelic genes of allotypic expression." Trans. Biochem. Soc. 4, 41-44 (1976).

23. Cannon, L.E., Margolies, M.N., Strosberg, A.D., Chen, F.W., Newell, J., and Haber, E. "Diversity among rabbit antibody light chain amino terminal sequence: a computer analysis." J. Immunol. 117, 160-167 (1976).

24. Van Hoegaerden, M. and Strosberg, A.D. "Partial amino acid sequence in the N-terminal region of an anti-*Micrococcus lysodeikticus* antibody heavy chain of allotype a1." FEBS Letters 66, 35-38 (1976).

25. Vauquelin, G., Lacombe, M.L., Guellaen, G., Strosberg, A.D., and Hanoune, J. "Tazolol (1-isopropylamino-3-(2-thiazoloxy)-2-propanol) as a ß-adrenergic blocker." Biochem. Pharmacol. 25, 2605-2608 (1976).

26. Vauquelin, G., Verheyden, R., Ebinger, G., and Strosberg, A.D. "Chemical aspects of the deazotation of catecholamines derivatives and metabolites for urinary screening tests." Clinic. Chem. 22, 1955-1961 (1976).

27. Chen, F.W., Cannon, L.E., Margolies, M.N., Strosberg, A.D., and Haber, E. "Purification, specificity and hypervariable region sequence of anti-pneumococcal polysaccharide antibodies elicited in a single rabbit." J. Immunol., 117, 807-813 (1976).

28. Van Driessche, E., Foriers, A., Strosberg, A.D., and Kanarek, L. "N-terminal sequences of the a and b subunits of the lectin from the garden pea (*Pisum sativum*)." FEBS Letters 71, 220-223 (1976).

29. Strosberg, A.D., Van Hoegaerden, M., and Schreiber, A. "Evolution de la réponse immunitaire contre la bactérie *Micrococcus lysodeikticus*." Ann. d'Immunol. (Inst. Pasteur) 128C, 345-349 (1977).

30. Strosberg, A.D. and Janssen, L. "L'évolution de la chaîne légère kappa d'IgG du lapin: multiple substitutions entre formes allèles." Ann. d'Immunol. (Inst. Pasteur) 128C, 351-353 (1977).

31. Strosberg, A.D. "Multiple expression of rabbit allotypes: the tip of the iceberg." Immunogenetics 4, 499-513 (1977).

32. Foriers, A., Wuilmart, C., Van Driessche, E., De Neve, R., Kanarek, L. and Strosberg, A.D. "The subunit structure and N-terminal sequences of the a and ß subunits of the lentil lectin (*Lens culinaris*)." FEBS Letters 75, 237-240 (1977).

33. Foriers, A., Wuilmart, C., Sharon, N., and Strosberg, A.D. "Extensive sequence homologies among lectins from leguminous plants." Biochem. Biophys. Res. Comm. 75, 980-986 (1977).

34. Gigot, D., Glansdorff, N., Legrain, C., Pierard, A., Stalon, V., Konigsberg, W., Caplier, I., Strosberg, A.D. and Hervé, G. "Comparison of the N-terminal sequences of aspartate and ornithine carbamyltransferases of *Escherichia coli*." FEBS Letters 81, 28-32 (1977).

35. Vauquelin, G., Geynet, P., Hanoune, J., and Strosberg, A.D. "Isolation of adenylate cyclase-free, ß-adrenergic receptor from turkey erythrocyte membranes by affinity chromatography." Proc. Natl. Acad. Sci. USA 74, 3710-3714 (1977).

36. Closset, J., Maghuin-Rogister, G., Hennen, G., and Strosberg, A.D. "Porcine follitropin. The amino acid sequence of the ß-subunit." Eur. J. Biochem. 86, 115-120 (1978).

37. Guellaen, G., Yates, M., Vauquelin, G., Strosberg, A.D. and Hanoune, J. "Characterization with [^{3}H]-dihydroergocryptine of the a-adrenergic receptor of the hepatic plasma membrane: comparison with the ß-adrenergic receptor in normal and adrenalectomized rats." J. Biol. Chem. 253, 1114-1120 (1978).

38. Zeeuws, R., and Strosberg, A.D. "The use of methanol in high performance liquid chromatography of phenylthiohydantoin-amino acids." FEBS Letters 85, 68-72 (1978).

39. Schreiber, A.B., Strosberg, A.D., and Pecht, I. "Fluorescence of tryptophanyl residues in homogeneous rabbit antibodies : variability in quantum yields and degree of exposure to solvent." Immunochemistry 15, 207-212 (1978).

40. Hoebeke, J., Vauquelin, G., and Strosberg, A.D. "The production and characterization of antibodies against ß-adrenergic antagonists." Biochem. Pharmacol., 27, 1527-1532 (1978).

41. Foriers, A., De Neve, R., Kanarek, L., and Strosberg, A.D. "A common ancestor for concanavalin A and lentil lectin?" Proc. Natl. Acad. Sci. USA 75, 1136-1139 (1978).

42. Mandy, W.J., and Strosberg, A.D. "Latent expression of a Cg gene." J. Immunol. 120, 1160-1163 (1978).

43. Schreiber, A.B., Hoebeke, J., Bergman, Y., Haimovich, J., and Strosberg, A.D. "A quantitative fluorometric assay for detection and characterization of Fc receptors." J. Immunol. 121, 19-23 (1978).

44. Van Hoegaerden, M., and Strosberg, A.D. "Sequence of a rabbit anti-micrococcus lysodeikticus antibody light chain." Biochemistry 17, 4311-4317 (1978).

45. Hoebeke, J., Foriers, A., Schreiber, A.B., and Strosberg, A.D. "Equilibrium and studies of the binding of *lens culinaris* lectin to rabbit erythrocytes by a quantitative fluorometric method." Biochemistry 17, 5000-5005 (1978).

46. Vray, B., Hoebeke, J., Zeeuws, R., and Strosberg, A.D. "Induction of anti-phosphorylcholine antibodies of restricted heterogeneity in rabbits." Immunochemistry 15, 869-874 (1978).

47. Vauquelin, G., Geynet, P., Hanoune, J., and Strosberg, A.D. "Purification of ß-adrenergic receptors : the search for an adequate affinity absorbent." Life Sciences 23, 1791-1796 (1978).

48. Vrijsen, R., Boeye, A. and Strosberg, A.D. "Amino-terminal sequence ambiguity in three capsid polypeptides of poliovirus." Biochem. Biophys. Res. Comm. 85, 1596-1601 (1978).

49. Strosberg, A.D., Emorine, L. and Zeeuws, R. "The structural polymorphism of the rabbit and other lagomorph allotypes constitute evidence for a control mechanism regulating the expression of closely linked duplicated genes." Ann. Immunol. (Inst. Pasteur) 130C,157-166 (1979).

50. Vauquelin, G., Bottari, S., Kanarek, L., and Strosberg, A.D. "Evidence for essential disulfide bonds in $ß_1$-adrenergic receptors of turkey erythrocyte membranes." J. Biol. Chem. 254, 4462-4469 (1979).

51. Bottari, S., Vauquelin, G., Durieu, O., Klutchko, C., and Strosberg, A.D. "The ß-adrenergic receptor of turkey erythrocyte membranes: conformational modification by ß-adrenergic agonists." Biochem. Biophys. Res. Comm. 86, 1311-1318 (1979).

52. Foriers, A., De Neve, R. and Strosberg, A.D. "Lectin sequences as a tool for chemotaxonomical classification." Phys. Végétale 17, 597-606 (1979).

53. Baumann, C., Rudiger, H., and Strosberg, A.D. "A comparison of the two lectins from *Vicia cracca*." FEBS Letters 102, 216-218 (1979).

54. Emorine L., Dutka, S., Paroutaud, P., and Strosberg, A.D. "The structural correlates of the rabbit light chain b allotypes: sequence studies of b5 and b6 chains." Mol. Immunol. 16, 997-1004 (1979).

55. Hoebeke, J., Vray, B., Foriers, A., and Strosberg, A.D. "Redistribution of lentil lectin receptors on rabbit plymorphonuclear membranes." Proc. Prot. Biol. Fluids 27, 443-446 (1979).

56. Vauquelin, G., Geynet, P., Hanoune, J., and Strosberg, A.D. "Affinity chromatography of the ß-adrenergic receptor of the turkey erythrocyte membrane." Eur. J. Biochem. 98, 543-556 (1979)

57. Seman, F., Dognin, M.J., Stanislawski, M., Seman, M. and Strosberg, A.D. "Structural studies of murine allotype: multiple substitutions between g2a allotype Ig-1a and Ig-1b." Immunol. Lett. 1 , 141-144 (1979).

58. Vray B., Hoebeke, J., St-Guillain, M., Leloup, R., and Strosberg, A.D. "A new quantitative fluorimetric assay for phagocytosis of bacteria." Scand J. of Immunol. 11, 147-153 (1979).

59. Devillers-Thiery, A., Changeux, J.P., Paroutaud, P., and Strosberg, A.D. "The amino-terminal sequence of the 40 000 molecular weight subunit of the acetylcholine receptor protein from *Torpedo marmorata*." FEBS Letters 104, 99-105 (1979).

60. Vauquelin, G., Bottari, S., and Strosberg, A.D. "Inactivation of the β-adrenergic receptors by N-ethylmalate cyclase activation." Mol. Pharmacol. 17, 163-171 (1980).

61. Strosberg, A.D., Vauquelin, G., Durieu-Trautmann, O., Delavier-Klutchko, C., Bottari, S. and André, C. "Towards the chemical and functional characterization of the ß-adrenergic receptor: a review." Trends in Biochem. Sci. 5, 11-14 (1980).

62. Schreiber, A.B., Hoebeke, J., Vray, B., and Strosberg, A.D. "Evidence for reversible microclustering of lentil lectin membrane receptors on HeLa cells." FEBS Letters 111, 303-306 (1980).

63. Vauquelin, G., Bottari, S., André, C., Jacobsson, B., and Strosberg, A.D. "Interaction between β-adrenergic receptors and guanine nucleotide sites in turkey erythrocyte membranes." Proc. Natl. Acad. Sci. USA 77, 3801-3805 (1980).

64. Durieu-Trautmann, O., Delavier-Klutchko, C., Vauquelin, G., and Strosberg, A.D. "Visualization of the turkey erythrocyte ß-adrenergic receptor." J. Supra. Struc. 13, 411-419 (1980).

65. Schreiber, A.B., Couraud, P.O., André, C., Vray, B., and Strosberg, A.D. "Anti-alprenolol anti-idiotypic antibodies bind to ß-adrenergic receptors and modulate catecholamine sensitive adenylate cyclase." Proc. Natl. Acad. Sci. USA 77, 7385-7389 (1980).

66. Delavier-Klutchko, C., Durieu-Trautmann, O., Couraud, P.O., André, C., and Strosberg, A.D. "Solubilization of a catecholamine sensitive guanosine triphosphatase from turkey erythrocyte membranes." FEBS Letters 117, 341-343 (1980).

67. Emorine, L. and Strosberg, A.D. "Allotype associated J regions in rabbit light chains." Immunol. Lett. 2, 107-109 (1980).

68. Schreiber, A.B., Lambermont, M., Strosberg, A.D. and Wybran, J. "A fluorometric assay for red blood cell antibodies". Tansfusion 21, 178-183 (1981).

69. Schreiber, A.B., Hoebeke, J., Vray, B. and Strosberg, A.D. "Resonance energy transfer studies of the mechanisms of microclustering of lentil lectin membrane receptors on HeLas cells". Exp. Cell Res. 132, 273-280 (1981).

70. Jacobsson, B., Vauquelin, G., Weslau, C., Smith, U., and Strosberg, A.D. "Catecholamine-induced desensitization of ß-adrenergic receptors and adenylate cyclase in human adipose cells". Eur. J. Biochem. 228, 1-6 (1981).

71. Foriers, A., Lebrun, E., Van Rapenbusch, R., de Neve, R., and Strosberg, A.D. "The structure of lentil (*Lens culinaris*) lectin". J. Biol. Chem 256, 5550-5560 (1981).

72. Petit-Koskas, E., Schreiberg, A.B., Favre, C., Chemla, R., Vray, B., Zurawski, V., Buttin, G., Cazenave, P.-A., and Strosberg, A.D. "Switched allotype expression in an immunoglobulin-non secreting rabbit lymphoïd cell line fused with rabbit gangliocytes". Eur. J. Immunol. 11, 388-392 (1981).

73. Strosberg, A.D., Couraud, P.-O., and Schreiber, A.B. "Immunological studies of hormone receptors : a two-way approach". Immunol. Today 10, 75-79 (1981).

74. Dogin, M.J., Lauwereys, M., and Strosberg, A.D. "Multiple amino acid substitutions between murine g2a heavy chain Fc regions of Ig1a and Ig1b allotypic forms". Proc. Natl. Acad. Sci. USA 78, 4031-4035 (1981).

75. Couraud, P.-O., Delavier-Klutchko, C., Durieu-Trautmann, O., and Strosberg, A.D. "Antibodies raised against ß-adrenergic receptors stimulate adenylate cyclase". Biochem. Biophys. Res. Comm. 99, 1295-1302 (1981).

76. André, C., Vauquelin, G., de Backer, J.-P., and Strosberg, A.D. "Identification and chemical characterization of ß-adrenergic receptors in intact turkey erythrocytes". Biochem. Pharmacol. 30, 2787-2795 (1981).

77. Bouhnik, J., Clauser, E., Strosberg, A.D., Fresnoy, J.-P., Ménard, J., and Corvol, P. "Rat angiotensinogen and desangiotensin I. Angiotensinogen : purification, characterization and partial sequencing". Biochemistry 20, 7010-7015 (1981).

78. Baumann, C.M., Strosberg, A.D., and Rudiger, H. "Purification and characterization of a mannose/glucose specific-lectin from *Vicia cracca*." Eur. J. Biochem. 122, 105-110 (1982).

79. Strosberg, A.D., Couraud, P.O., Durieu-Trautmann, O., and Delavier-Klutchko, C. "Biochemical and immunochemical analysis of ß-adrenergic receptor adenylate cyclase complexes". Trends in Pharmacol. Sci. 3, 282-285 (1982).

80. Vauquelin, G., André, C., de Backer, J.-P., Laduron, P., and Strosberg, A.D. "Agonist mediated conformational changes of muscarinic receptors in rat brain". Eur. J. Biochem. 125, 117-124 (1982).

81. Panthier, J.-J., Foote, S., Chambraud, B., Strosberg, A.D., Corvol, P., and Rougeon, F. "Complete amino acid sequence and maturation of the mouse submaxillary gland renin precursor". Nature 298, 90-92 (1982).

82. André, C., Vauquelin, G., Severne, Y., de Backer, J.-P., and Strosberg, A.D. "Dual effect of N-ethylmaleimide on agonist-mediated confomational changes of ß-adrenergic receptors". Biochemical. Pharmacol. 31, 3657-3662 (1982).

83. Strosberg, A.D., Couraud, P.O., Delavier-Klutchko, C., Durieu-Trautmann, O., Schmutz, A., and Lü, B.-Z. "Immunologie des récepteurs ß-adrénergiques : un modèle d'étude d'une image hormonomimétique". Ann. Immunol. 133D, 191-197 (1982).

84. Vauquelin, G., Cech, S.Y., André, C., Strosberg, A.D., and Maguire, M.E. "Distinctions in ß-adrenergic receptor interactions with the magnesium-guanine nucleotide coupling proteins in turkey erythrocyte and S49 lymphoma membranes". J. Cyclic Nucl. Res., 8, 149-162 (1982).

85. Ayadi, H., Paroutaud, P., Benammar, A., Cazenave, P.A., and Strosberg, A.D. "Structural studies of a wild rabbit immunoglobulin light chain constant region." Mol. Immunol. 20, 223-227 (1983).

86. Deschodt-Lanckman, M., Bui, N.D., Koulischer, D., Paroutaud, P. and Strosberg, A.D. "Cholecystokinin Octa- and Tetrapeptide Degradation by Synaptic Membranes. II. Solubilization and Separation of Membrane-Bound CCK-8 Cleaving Enzymes". Peptides, 4, 71-78 (1983).

87. Ayadi, H., Dutka, S., Paroutaud, P., and Strosberg, A.D. "The amino acid sequence of a rabbit immunoglobulin light chain of allotype b5." Biochemistry, 22, 993-998 (1983).

88. Strosberg, A.D. "Anti-idiotype and Anti-Hormone Receptor Antibodies" In "Springer Seminars in Immunopathology" 6, 67-78 (1983).

89. André, C., De Backer, J.P., Guillet, J.G., Vanderheyden, P., Vauquelin, G. and Strosberg, A.D. "Purification of muscarinic acetylcholine receptors by affinity chromatography." EMBO J. 2 4, 499-504 (1983).

90. Couraud, P.O., Lü, B.-Z., and Strosberg, A.D. "Cyclical anti-idiotypic response to antihormone antibodies due to neutralization by autologous anti-anti-idiotype antibodies which bind hormone." J. Exp. Med. Vol.157, 1369-1378 (1983).

91. Lebrun, E., Foriers, A., Hoebeke, J., Strosberg, A.D., and Van Rapenbusch, R. "Crystallization and preliminary X-ray diffraction studies of the mitogenic lentil (*Lens culinaris*) lectin." J. Mol. Biol. 166, 99-100 (1983).

92. Deschodt-Lanckman, M., and Strosberg, A.D. "In vitro degradation of the C-terminal octapeptide of cholecystokinin by 'enkephalinase A'." FEBS Lett. 152, 109-113 (1983).

93. Couraud, P.O., Lü, B.Z., Schmutz, A., Durieu-Trautmann, O., Klutchko-Delavier, C., Hoebeke, J., and Strosberg, A.D. "Immunological studies of ß-adrenergic receptors." J. Cell. Biochem. 21, n.3, 187-193 (1983).

94. Guillet, J.-G., Hoebeke, J., Tram, C., Marullo, S., and Strosberg, A.D. "Monoclonal antibodies against Legionella pneumophila : Antibody characterization, serological specificity and diagnostic possibilities." J. Clin. Microbiol. 18, 793-797 (1983).

95. Guillet, J.G., Marche, P., Hoebeke, J., and Strosberg, A.D. "Physiochemical studies on the antigen antibody complexes of two monoclonal antibodies against rabbit thymocytes." Mol. Immunol. 20, 1059-1068 (1983).

96. Lü, B.-Z., Couraud, P.O., Schmutz, A., and Strosberg, A.D. "The internal image of catecholamines: expression and regulation of a functional network." Ann. N.Y. Acad. Sci. 418, 240-247 (1984).

97. André, C., Guillet, J.G., De Backer, J.P., Vanderheyden, P., Hoebeke, J. and Strosberg, A.D. "Monoclonal antibodies against muscarinic acetylcholine receptors recognize active and denatured forms". EMBO J. 3, 17-21 (1984).

98. Hoebeke, J., Durieu, O., Delavier, C., Schmutz, A. and Strosberg, A.D. "Biochemical and immunological studies of ß-adrenergic receptors on various cell types". Adv. Cyclic. Nucl. Res. 17, 73-80 (1984).

99. Hoebeke, J., Chamat, S., Marullo, S., Guillet, J.-G. et Strosberg, A.D. "The monoclonal antibodies as pharmacological agents : diagnostic and therapeutic advantage". Bio-Sciences 3, 12-15 (1984).

100. Strosberg, A.D. "Receptors and Recognition: from ligand binding to gene structure". Trends in Biochem. Sci. 4, 166-169 (1984).

101. Chamat, S., Hoebeke, J. and Strosberg, A.D. "Monoclonal antibodies specific for ß-adrenergic ligands". J. Immunol. 133, 1547-1552 (1984).

102. Leiber, D. and Harbon, S., Guillet, J.G., André, C. and Strosberg, A.D. "Monoclonal antibodies to purified muscarinic receptor display agonist-like activity". Proc. Natl. Acad. Sci. U.S.A. 81, 73-80 (1984).

103. Delavier-Klutchko, C., Hoebeke, J. and Strosberg, A.D. "The human carcinoma cell line A431 possesses large numbers of functional ß-adrenergic receptors" FEBS Letters 169, 151-155 (1984).

104. Guillet, J.-G., Chamat, S., Hoebeke, J. and Strosberg, A.D. "Production and detection of monoclonal anti-idiotype antibodies directed against a monoclonal anti-ß-adrenergic ligand antibody" J. Immunol. Methods, 74, 163-171 (1984).

105. Venter, J.C., Berzofsky, J.A., Lindstrom, J., Jacobs, S., Fraser, C.M., Kohn, L.D., Schneider, W., Greene, G.L., Strosberg, A.D. and Erlanger, B.F. "Monoclonal and anti-idiotypic antibodies as probes for receptor structure and function" Fed. Proc. 43, 2532-2539 (1984).

106. Vo-Quang, T., Malpiece, Y., Buffard, D., Kaminski, P.-A., Vidal, D. and Strosberg, A.D. "Rapid large-scale purification of plasmid DNA by medium or low pressure gel filtration. Application: construction of thermoamplifiable expression vectors" Bioscience Reports 5, 2, 101-111 (1985).

107. Durieu-Trautmann, O., Delavier-Klutchko, C., Hoebeke, J. and Strosberg, A.D. "Altered binding properties of ß-adrenergic receptors and lack of coupling to adenylate cyclase in P815 mastocytoma cells" Eur. J. Pharmacol. 108, 133-141 (1985).

108. Guillet, J.-G., Kaveri, S., Durieu-Trautmann, O., Delavier-Klutchko, C., Hoebeke, J. and Strosberg, A.D., "ß-adrenergic agonist activity of monoclonal anti-idiotypic antibody" Proc. Natl. Acad. Sci. USA 82, 1781-1784 (1985).

109. Strosberg, A.D., Chamat, S., Guillet, J.-G., Lavaux, B., Emorine, L. and Hoebeke, J. "Idiotypy of catecholamine binding proteins" Ann. Immunol. (Inst. Pasteur) 136C, 157-168 (1985).

110. Cervantes-Olivier, P., Durieu-Trautmann, O., Delavier-Klutchko, C. and Strosberg, A.D. "The oligosaccharide moiety of the $ß_1$-adrenergic receptor from turkey erythrocytes has a bi-antennary, N-acetyl lactosamine containing structure" Biochemistry 24, 3765-3770 (1985).

111. Marullo, S., Hoebeke, J., Guillet, J.G. and Strosberg, A.D. "Structural analysis of the epitope recognized by a monoclonal antibody directed against tricyclic antidepressants". J. Immunol.135,1, 471-477 (1985).

112. Hoebeke, J. "La diversité des anticorps : une leçon d'économie moléculaire". Courrier du CNRS 58, 10-13 (1985).

113. Do Ngoc, L., Paroutaud, P., Dunia, I., Benedetti, E.L. and Hoebeke, J. "Sequence analysis of peptide fragments from the intrinsic membrane protein of calf lens fibers MP26 and its natural maturation product MP22". FEBS Letters 181, 74-78 (1985).

114. Vallon, O., Dunia, I., Favard-Sereno, C., Hoebeke, J. and Benedetti, E.L. "MP26 in the bovine lens : a post-embedding immunocytochemical study". Biology of the Cell 53, 85-88 (1985).

115. Lauwereys, M., Foriers, A., Sharon, N. and Strosberg, A.D. "Sequence studies of peanut agglutinin" FEBS Letters 181, 241-244 (1985).

116. Strosberg, A.D., Guillet, J.G., Chamat, S. and Hoebeke, J. "Recognition of physiological receptors by anti-idiotypic antibodies : molecular mimicry of the ligand or cross reactivity?" Curr. Topics Microbiol. Immunol. 119, 93-112 (1985).

117. Strosberg, A.D. "Monoclonal antibodies as tools for the study of membrane receptors" Biochem. Soc. Trans. 13, 6, 1106-1107 (1985).

118. Chamat, S., Hoebeke, J., Emorine, L., Guillet, J.-G. and Strosberg, A.D. "The immune response towards ß-adrenergic ligands and their receptors VI. Idiotypy of monoclonal anti-alprenolol antibodies". J. Immunol. 136, 3805-3811 (1986).

119. Chapot, M.-P., Peumans, W.J. and Strosberg, A.D. "Extensive homologies between lectins from non-leguminous plants" FEBS Letters 1, 2, 231-234 (1986).

120. Denis, H., Marullo, S., Hoebeke, J. and Strosberg, A.D. "Enzyme linked immunosorbent assay for amitriptyline and other antidepressants using a monoclonal antibody" Clinica Chimica Acta 159, 257-267 (1986).

121. Pène, J., Rousseau, V., Zaghouani, H., Paroutaud, P., Strosberg, A.D. and Stanislawski, M. "Monoclonal anti-⊛ (1--3) dextran antibodies to Igh^a BALB/c and Igh^b C.B20 mice display striking similarities". J. Immunol. 137, 2319-2324 (1986).

122. Kaminski, P.A., Buffard, D. and Strosberg, A.D. "Detection of lectin-related sequences in the genome of *Sesbania rostrata*". Plant Science 46, 111-116 (1986).

123. Bon, S., Chang, J.-Y. and Strosberg, A.D. "Identical N-terminal peptide sequences of asymmetric forms of a low-salt-soluble and detergent-soluble amphiphilic dimers of Torpedo acetylcholinesterase" FEBS Lett. 209, 206-212 (1986).

124. Petitjean, F., Guillet, J.G., Vray, B., Strosberg, A.D. and Hoebeke, J. "Partial characterization of *Legionella pneumophila* sero group 1 immunodominant antigenic determinant recognized by a monoclonal antibody" Comp. Immunol. Microbiol. Inf. Dis. 1, 9-23 (1987).

125. Marullo, S., Hoebeke, J., Guillet, J.-G., André, C. and Strosberg, A.D. "Immunological mimicry by a monoclonal antibody of the tricyclic antidepressants' binding site on muscarinic acetylcholine receptors". J. Immunol. 138, 524-526 (1987).

126. Chapot, M.P., Cervantes, P., Kaveri, S., Durieu-Trautmann, O., Delavier-Klutchlo, C., Emorine, L., Couraud, P.O. and Strosberg, A.D. "Biochemical and immunochemical analysis of avian $ß_1$ and mammalian $ß_2$-adrenergic receptors". J. Receptor Res. 7, 1-15 (1987).

127. André, C., Marullo, S., Guillet, J.G., Lauwereys, M., Kaveri, S., Hoebeke, J. and Strosberg, A.D. "Immunochemical studies of the muscarinic acetylcholine receptor". J. Receptor Res. 7, 89-103 (1987).

128. Strosberg, A.D. "Molecular and functional properties of ß-adrenergic receptors". J. Cardiol. 59, 3F-9F (1987).

129. Hoebeke, J., Kaveri, S.V. and Strosberg, A.D. "Immunology of muscarinic acetylcholine and ß-adrenergic catecholamine receptors". Acta Med. Scand., Suppl. 715, 107-110 (1987).

130. Kaveri, S.V., Cervantes-Olivier, P., Delavier-Klutchko, C. and Strosberg, A.D. "Monoclonal antibodies directed against the human A431 $ß_2$-adrenergic receptor recognize two major polypeptide chains" Eur. J. Biochem. 167, 449-456 (1987).

131. Raposo, G., Marullo, S., Dunia, I., André, C., Guillet, J.-G., Strosberg, A.D., Benedetti, E.L. and Hoebeke, J. "Redistribution of muscarinic acetylcholine receptors on CCL137 fibroblasts induced by regulatory ligands" Biol. Cell 60, 117-124 (1987).

132. Hoebeke, J., Engelborghs, Y., Chamat, S. and Strosberg, A.D. "The immune response towards ß-adrenergic ligands and their receptors : VII. Equilibrium and kinetic binding studies of L-alprenolol to a monoclonal anti-alprenolol antibody". Mol. Immunol. 6, Vol. 24, 621-629 (1987).

133. Paroutaud, P., Levi, G., Aberdam, D., Berkovitz, Z., Teichberg, V.I. and Strosberg, A.D. "Extensive amino acid sequence homologies between animal lectins". Proc. Natl. Acad. Sci. USA 84, 6345-6348 (1987).

134. Kaveri, S.V., Frémy, J.M., Lapeyre, C. and Strosberg, A.D. "Immunodetection and immuno-purification of aflatoxins using a high affinity monoclonal antibody to aflatoxin B1". Lett. Applied Microbiol. 4, 71-75 (1987).

135. Lapeyre, C., Kaveri, S.V., Janin, F. and Strosberg, A.D. "Production and characterization of monoclonal antibodies to staphylococcal enterotoxins : use in immunodetection and immuno-purification". Mol. Immunol. 24, 1243-1254 (1987).

136. Emorine, L.J., Marullo, S., Delavier-Klutchko, C., Kaveri, S.V., Durieu-Trautmann, O. and Strosberg, A.D. "Structure of the gene for the human ß$_2$-adrenergic receptor : expression and promoter characterization". Proc. Natl. Acad. Sci. USA 84, 6995-6999 (1987).

137. Lapeyre, C., Kaveri, S.V. and Strosberg, A.D. "A novel approach to prevent the interference of protein A in immunoassays of enterotoxins". Lett. Applied Microbiol. 5, 55-59 (1987).

138. Kaminski, P.A., Buffard, D. and Strosberg, A.D. "The pea lectin gene family contains only one functional gene". Plant Mol. Biol. 9, 497-507 (1987).

139. Buffard, D., Kaminski, P.A. and Strosberg, A.D. "Lectin gene expression in Pea (*Pisum sativum*) roots". Planta 173, 367-372 (1988).

140. André, C., Marullo, S., Convents, A., Lauwereys, M., Guillet, J.-G., Hoebeke, J. and Strosberg, A.D. "A human embryonic lung fibroblast with a high density of muscarinic acetylcholine receptors". Eur. J. Biochem. 171, 401-407 (1988).

141. Cervantes-Olivier, P., Delavier-Klutchko, C., Durieu-Trautmann, O., Kaveri, S., Desmandril, M. and Strosberg, A.D. "The ß$_2$-adrenergic receptors of the human epidermoid carcinoma cells bear two different types of oligosaccharides which influence expression on the cell surface". Biochem. J. 250, 133-143 (1988).

142. Nahmias, C., A.D. Strosberg, Emorine, L.J. "The immune response towards ß-adrenergic ligands and their receptors. VIII. Extensive diversity of V_H and V_L genes encoding anti-alprenolol antibodies". J. Immunol. 140, 1304-1311 (1988).

143. Matsuyama, T., Luiten, P.G.M., Spencer Jr., D.G. and Strosberg, A.D. "Ultrastructural localization of immunoreactive sites for muscarinic acetylcholine receptor proteins in the rat cerebral cortex". Neurosci. Res. Comm. 2, 69-76 (1988).

144. Moummi, C., Magous, R., Strosberg, A.D. and Bali, J.P. "Muscarinic receptors in isolated smooth muscle cells from gastric antrum". Biochem. Pharmacol. 37, 1363-1369 (1988).

145. Chouchane, L., Strosberg A.D. and Hoebeke J. "Stereospecific immuno-recognition of the tetracyclic anti-depressant oxaprotiline". Mol. Immunol. 25, 1299-1308 (1988).

146. Cazaubon, S., Couraud, P.O., Hoebeke, J., Nahmias, C., Emorine, L.J., Grass-Masse, H. and Strosberg, A.D. "Anti-HV3 peptide antibodies as probes for conformational changes in immunoglobulins" J. Immunol. Methods 114 13-20 (1988).

147. Marullo, S., Delavier-Klutchko, C., Eshdat, Y., Strosberg, A.D. and Emorine, L. "Human ß$_2$-adrenergic receptors expressed in *Escherichia coli* membranes retain their pharmacological properties". Proc. Natl. Acad. Sci. USA 85, 7551-7555 (1988).

148. Mûller-Marschhausen, U., Grothe, C., Kaveri, S., Strosberg, A.D., Verhofstad, A.A.J. and Unsicker, K. "Catecholaminergic nerves in the embryonic chick ovary: co-localization with ß2-adrenoceptor-bearing steroidogenic cells". Cell Tissue Res 254 , 1-9 (1988).

149. Luiten, P.G.M., Wouterlood, F.G., Matsuyama, T., Strosberg, A.D., Buwalda, B. and Gaykema, R.P.A. "Immunocytochemical applications in neuroanatomy". In Histochemistry 90, Springer Verlag, 85-97 (1988).

150. Strosberg, A.D. "Anti-idiotypic antibodies that interact with the ß-adrenergic catecholamine receptor". In " Antibodies, Antigens, and Molecular Mimicry", Ed. J.J. Langone, Meth. Enzymol. 178, 265-274 (1989).

151. Strosberg, A.D. "Interaction of antidiotypic antibodies with membrane receptors". In " Antibodies, Antigens, and Molecular Mimicry", Ed. J.J. Langone, Meth. Enzymol. 178, 179-190 (1989).

152. Lamacz, M., Tonon, M.C., Louiset, E., Cazin, L., Strosberg, A.D. and Vaudry, H. "Acetylcholine stimulates ⊕-MSH release from frog pituitary melanotrophs through activation of muscarinic and nicotinic receptors" Endocrinol. 125, 707-714 (1989).

153. Nahmias, C., Cazaubon, S. and Strosberg A.D. "A rabbit antiserum detects a Vh J558 subgroup marker highly expressed among anti-alprenolol antibodies" J. Immunol. 142, 871-876 (1989).

154. Eshdat, Y., Chapot, M.-P. and Strosberg, A.D. "Chemical characterization of ligand binding site fragments from turkey ß-adrenergic receptor". FEBS Lett. 246, 166-170 (1989).

155. Cazaubon, S., Marais, R., Parker, P., and Strosberg A.D.:"Monoclonal antibodies to protein Kinase C⊕ functional relationship between epitopes and cofactor binding sites". Eur. J. Biochem. 182, 401-406 (1989).

156. Strosberg, A.D., Camoin, L., Guillaume, J.L. "Microsequencing of peptides and proteins". Trends Anal.Chem.Sci. 8, 292-298 (1989).

157. Emorine, L.J., Marullo, S., Briend-Sutren, M.M.,Patey, G., Tate, K., Delavier-Klutchko, C. and Strosberg, A.D. "Molecular characterization of a new human ß-adrenergic receptor involved in catecholamine control of metabolism". Science 245, 1118-1121 (1989).

158. Marullo, S., Delavier-Klutchko, C., Guillet, J.-G., Charbit, A., Strosberg, A.D. and Emorine, L.J. "Expression of human ß1- and ß2-adrenergic receptors in *E. coli* as a new tool for ligand screening". Bio/Technology 7, 923-927 (1989).

159. Magnusson, Y., Höyer, S., Lengagne, R., Chapot, M.P., Guillet, J.G., Hjalmarsson, A., Strosberg, A.D. and Hoebeke, J. "Antigenic analysis of the second extra-cellular loop of the human ß-adrenergic receptors" Clin. Exp. Immunol. 78, 42-48 (1989).

160. Chouchane, L., Goosens, D., Rouger, Ph. and Strosberg, A.D. "Amino acid sequence of the variable domains of a human anti-Rhesus (c) antibody : presence of an unusually long CDR3 in the l chain" Mol. Immunol. 26 (1989).

161. Mazza, G., Nahmias, C., Strosberg, A.D., and Fougereau, M. "Idiotypic cross-reactivity of anti-GAT and anti-alprenolol antibodies: an approach to the structural correlates of the pGAT idiotypic specificity". Mol. Immunol. 26, 827-833 (1989).

162. Raposo, G., Dunia, I., Benedetti, L. and Strosberg, A.D. "Internalization of ß-adrenergic receptor in A431 cells involves non-coated vesicles". Eur. J. Cell Biol. 50, 2 (1989).

163. Chapot, M.P., Couraud, P.O., Schmutz, A.,and Strosberg, A.D. "A monoclonal antibody directed against the ß1-adrenergic receptor from turkey erythrocyte membranes". Hybridoma 8, 835-843 (1989).

164. Lebrun, E., Davoust, D., Hennig, Ph. Strosberg, A.D. and Van Rapenbusch, R. "Proton assignment and conformational analysis of the Hv3 peptide segment from monoclonal anti-alprenolol antibody 37A4". Peptide and Protein Res. (in press) (1990).

165. Jardon, B., Sahel, J., Strosberg, A.D., Roussel, G., Yücel, H. and Bonaventure, N. "Immuno-cytochemical localization of muscarinic receptors in the frog retina: physiological correlates". Visual Neurosci. (submitted) (1990).

166. Chapot, M.P., Eshdat, Y., Marullo, S., Guillet, J.G., Charbit, A., Strosberg, A.D. and Delavier-Klutchko, C. "Localization and characterization of three different vertebrate ß-adrenergic receptors expressed in *E. coli* as fusion proteins with the outer membrane protein lamb". Eur. J. Biochem. 187, 137-144 (1990).

167. Marullo, S., Emorine, L.J., Strosberg, A.D. and Delavier-Klutchko, C. "Selective binding of ligands to ß1/ß2 or chimeric ß1/ß2-adrenergic receptors involves multiple subsites". EMBO J. 9, 1471-1476 (1990).

168. Moreau, T., Hoebeke, J., Lalmanach, G., Hattab, M. & Gauthier, F. "Simulation of the inhibitory cystatin surface by a synthetic peptide". Biochem. J. 167, 117-122 (1990).

169. Chouchane, L., Breyer, J., Van Spronsen, A., Guillaume, J.-L., Goossens, D., Rouger, Ph. & Strosberg, A.D. "Molecular genetics of human anti-Rh antibodies". Devel. Biol. Standar. (in press) (1990).

170. Khang, N.Q., Guillaume, J.L. & Hoebeke, J. "A blood group A specific lectin from the seeds of *Crotalaria striata*". Biochim. Biophys. Acta 1033, 210-213 (1990).

171. Durieu-Trautmann, O., Foignant, N., Strosberg, A.D. & Couraud, P.O. "Coexpression of ß1- and ß2-adrenergic receptors on bovine brain capillary endothelial cells in culture". J. Neurochem. (in press) (1990).

172. Fève, B., Emorine, L.J., Briend-Sutren, M.-M., Lasnier, F., Strosberg, A.D. & Pairault, J. "Differential regulation of b_1- and b_2-adrenergic receptor protein and mRNA levels by glucocorticoids during 3T3-F442A adipose differentiation". J. Biol. Chem. 265, 16343-16349 (1990).

173. Schröder, H., Zilles, K., Luiten, P.G.M. & Strosberg, A.D. "Immunocytochemical visualization of muscarinic cholinoceptors in the human cerebral cortex". Brain Research 514, 249-258 (1990).

174. Chouchane, L., Bringman, T., Barbier, S., Traincard, F. & Strosberg, A.D. "Targeted killing of yeast expressing a HIV-1 peptide by antibody-conjugated glucose oxidase and horseradish peroxidase". Immunol. Lett. 25, 359-366 (1990).

175. Breyer, R.M., Strosberg, A.D. & Guillet, J.-G. "Mutational analysis of ligand binding activity of ß2-adrenergic receptor expressed in *Escherichia coli*". EMBO J. 9, 2679-2684 (1990).

176. Petitjean, F., Dournon, E., Strosberg, A.D. & Hoebeke, J. "Isolation, purification and partial analysis -of the lipopolysaccharide antigenic determinant recognized by a monoclonal antibody to *Legionella pneumophila* serogroup 1". Res. Microbiol., 141,1077-1094 (1990).

177. Marullo, S., Emorine, L.J., Strosberg, A.D. & Delavier-Klutchko, C. "Selective binding of ligands to β_1/β_2 or chimeric β_1/β_2-adrenergic receptors involves multiple subsites". EMBO J. 9, 1471-1476 (1990).

177. Chapot, M.P., Eshdat, Y., Marullo, S., Guillet, J.G., Charbit, A., Strosberg, A.D. & Delavier-Klutchko, C. "Localization and characterization of three different vertebrate β-adrenergic receptors expressed in *E. coli* as fusion proteins with the outer membrane protein lamb". Eur. J. Biochem. 187, 137-144 (1990).

178. Moreau, T., Hoebeke, J., Lalmanach, G., Hattab, M. & Gauthier, F. "Simulation of the inhibitory cystatin surface by a synthetic peptide". Biochem. Biophys. Res. Comm. 167, 117-122 (1990).

179. Khang, N.Q., Guillaume, J.L. & Hoebeke, J. "A blood group A specific lectin from the seeds of *Crotalaria striata*". Biochim. Biophys. Acta 1033, 210-213 (1990).

180. Koman, A., Durieu-Trautmann, O., Couraud, P.O., Strosberg, A.D. & Weksler, B.B. "Modulation of muscarinic receptor expression in human embryonic lung fibroblasts by platelet-derived growth factor" Biochem. J. 270, 409-412 (1990).

181. Chouchane, L., Bringman, T., Barbier, S., Traincard, F. & Strosberg, A.D. "Targeted killing of yeast expressing a HIV-1 peptide by antibody-conjugated glucose oxidase and horseradish peroxidase". Immunol. Lett. 25, 359-366 (1990).

182. Breyer, R.M., Strosberg, A.D. & Guillet, J.-G. "Mutational analysis of ligand binding activity of β2-adrenergic receptor expressed in *Escherichia coli*". EMBO J. 9, 2679-2684 (1990).

183. Schröder, H., Zilles, K., Luiten, P.G.M. & Strosberg, A.D. "Immunocytochemical visualization of muscarinic cholinoceptors in the human cerebral cortex" Brain Res. 514, 249-258 (1990).

184; Cazaubon, S., Webster, C., Camoin, L., Strosberg, A.D. & Parker, P. "Effector-dependent conformational changes in protein kinase Cγ through epitope mapping with inhibitory monoclonal antobodies" Eur. J. Biochem. 194, 799-804 (1990).

185. Magnusson, Y., Marullo, S., H_yer, S,, Waagstein, F., Andersson, B., Vahine, A., Guillet, J.G., Strosberg, A.D. Hjalmarson, A. & Hoebeke, J. " Mapping of a functional autoimmune epitope on the β1-adrenergic receptor in patients with idiopathic dilated cardiomyopathy". J. Clin. Invest. 86, 1658-1663 (1990).

186. Emorine, L.J., Feve, B., Pairault, J., Briend-Sutren, M.M., Marullo, S., Delavier-Klutchko, C. & Strosberg, A.D. "Structural basis for functional diversity of β_1-, β_2-, and b3-adrenergic receptors". Biochem. Pharmacol. 41, 853-859 (1991).

187. Tate, K.M., Briend-Sutren, M.M., Emorine, L.E., Delavier-Klutchko, C., Marullo, S. and Strosberg, A.D. "Expression of 3 human β-adrenergic receptor subtypes in transfected Chinese Hamster Ovary cells" Eur. J. Biochem. 196, 357-361 (1991).

188. Strosberg, A.D. "Structure function relationship of proteins belonging to the family of receptors coupled to GTP binding proteins" Eur. J. Biochem. 196, 1-10 (1991).

189. Strosberg, A.D. & Leysen, J.E. "Receptor based assays" Current Opinion in Biotechnology 2, 30-36 (1991).

190. Couraud, P.O. & Strosberg, A.D. "Anti-idiotypic antibodies against hormone and neurotransmitter receptors" Biochem. Soc. Trans. 19, 147-151 (1991).

191. Durieu-Trautmann, O., Foignant, N., Strosberg, A.D. & Couraud, P.O. "Coexpression of b_1- and β_2-adrenergic receptors on bovine brain capillary endothelial cells in culture". J. Neurochem. 56, 775-781 (1991).

192. Couraud, P.O., Durieu-Trautmann, O., NGuyen, D.L., Marin, P., Gilbert, F. & Strosberg, A.D. "Functional Endothelin-1 receptors in rat astrocytoma C6". Eur. J. of Pharmacol. 206, 191-198 (1991).

193. Marin, P., Delumeau, J.C;, Durieu-trautmann, O., Le Nguyen, D., Pr_mont, J., Strosberg, A.D. & Couraud, P.O. "Are several G proteins involved in the different effects of endothelin-1 in mouse striatal astrocytes ?" J. Neurochem. 56, 1270-1275 (1991).

194. Luyten, W.H.M.L., Pauwels, P.J., Moereels, H., Marullo, S., Strosberg, A.D. and Leysen, J.E. "Comparative study of the binding properties of cloned human b_1- and b_2-adrenergic receptors expressed in *Escherichia coli*. Drug Investigation 3, 3-12 (1991).

195. Van Der Zee, E. A., Streefland, C., Strosberg, A.D., Schr_der H. & Luiten, P.G.M."Colocalization of muscarinic and nicotinic receptors in cholinoceptive neurons of the suprachiasmatic region in young and aged rats". Brain Res. 542, 348-352 (1991).

196. Van Der Zee, E. A., Benoit, R., Strosberg, A.D. & Luiten, P.G.M. "Coexistence of muscarinic acetylcholine receptors and somatostatin in non-pyramidal neurons of the rat dorsal hippocampus" Brain Res. Bulletin 26, 343-351 (1991).

197. Chouchane, L., Breyer, J., Van Spronsen, A., Guillaume, J.-L., Goossens, D., Rouger, Ph. & Strosberg, A.D. "Molecular genetics of human anti-Rh antibodies". Devel. Biol. Standar. 71, 9-14 (1991).

198. Strosberg, A.D., Marullo, S. & Guillet, J.G. "Immunologie des récepteurs d'hormone" Médecine/Sciences 33-39 John Libbey Eurotext publishers, Paris (1991).

199. Guillet, J.G., Hoebeke, J., Lengagne, R., Tate, K., Borras-Herera, F., Strosberg, A.D. & Borras-Cuesta, F. "Haplotype specific homology scanning microcomputer program to predict T cell epitopes within proteins" J. Mol. Recogn. 4, 17-25 (1991).

200. Dandeu, J. P., Rabillon, J., Guillaume, J. L., Camoin, L., Lux, M. & David, B. Isolation and Purification of Cat Albumin from Cat Serum by Copper Ion Affinity Chromatography - Further Analysis of Its Primary Structure. J. of Chromatography 539, 475-484 (1991).

201. Freissmuth, M., Selzer, E., Marullo, S., SchÙtz, W. & Strosberg, A.D. "Expression of two human β-adrenergic receptors in E. coli : functional interaction, with two forms of Gs". Proc. Natl. Acad. Sci. USA 88, 8548-8552 (1991).

202. Durieu-Trautmann, O., Couraud, P.O., Foignant-Chaverot, N. & Strosberg, A.D. "cAMP-dependent down regulation of Endothelin 1 receptors on rat astrocytoma C6 cells" Neurosci. Lett. 131, 175-178 (1991).

203. Coutinho, G., Durieu-Trautmann, O., Strosberg, A.D. & Couraud, P-O "Catecholamines stimulate the IFNγ-induced class II MHC expression on bovine brain capillary endothelial cells. J. Immunol. 147, 2525-2529 (1991).

204. Durieu-Trautmann, O., Foignant-Chaverot, N., Perdomo, J., Gounon P., Strosberg, A.D. & Couraud, P.O. "Immortalization of brain capillary endothelial cells with maintenance of structural characteristics of the blood-brain barrier endothelium" In Vitro 27A, 771-778 (1991).

205. Nahmias, C., Blin, N., Elalouf, J-M., Mattei, M.G., Strosberg, A.D. & Emorine, L.J. "Molecular characterization of the mouse β3-adrenergic receptor : relationship with the atypical receptor of adipocytes" EMBO J. 10, 3721-3727 (1991).

206. Couraud, P-O., Durieu-Trautmann, O., Mahe, E., Marin, P., le NGuyen, D. & Strosberg, A.D."Comparison of binding characteristics of endothelin receptors on subpopulations of astrocytes". Life Science 49, 1471-1476 (1991).

207. Schröder, H., Giacobini, E., Struble, R.G., Luiten, P.G.M., van der Zee, E.A., Zilles, K. & Strosberg, A.D. "Muscarinic cholinoceptive neurons in the frontal cortex in Alzheimer's disease" Brain Res. Bull. 27, 701-706.

208. van der Zee, E.A., de Jong, G.I., Strosberg, A.D. & Luiten , P.G.M. "Parvalbumin-positive neurons in rat dorsal hippocampus contain muscarinic acetylcholine receptors" Brain Res. Bull. 27, 697-700 (1991).

209 Fève, B., Emorine, L.J., Lasnier, F., Blin, N., Baude, B., Nahmias, C., Strosberg, A.D. & Pairault J. "Atypical β-adrenergic receptor in 3T3-F442A adipocytes: Pharmacological and molecular relationship with the human β3-adrenergic receptor". J. Biol. Chem. 266, 20329-20336 (1991).

210. Strosberg, A.D., Marullo, S., Guillet, J-G. & Emorine, L.J. "Ing_nierie des r_cepteurs €-adrénergiques des catécholamines" dans Conception, S_lection , Production et Analyse des Protéines de la Nouvelle Génération, 14ème Colloque SFM, 161-169 (1991).

211. Emorine, L.J., F_ve, B., Pairault, J., Briend-Sutren, M.M., Nahmias, C., Marullo, S., Delavier-Klutchko, C. & Strosberg, A.D. "The human β3-adrenergic receptor : relationship with atypical receptors" Am. J. of Clin. Nutrition. 55, 215S-218S (1992).

212. Strosberg, A.D & Marullo, S. "Functional expression of G protein-coupled receptors in microorganisms" Tr. Pharmacol. Sci. 13, 95-98 (1992).

213. Strosberg, A.D. "Biotechnology of the β-adrenergic receptors" Molecul. Neurobiol. 4, 211-250 (1992).

214. Bertin, B., Freissmuth, M., Breyer, R., Schütz, W., Marullo, S. & Strosberg, A.D. "Functional Expression of the Human serotonin 5HT-1A Receptor in *Escherichia coli* : Ligand binding properties and interaction with recombinant G protein a-subunits" J. Biol. Chem. 267, 8200-8206. (1992).

215. Avrameas, A. Guillet, J-G., Chouchane, L., Moraillon, A., Sonigo, P. & Strosberg, A.D. "Localization of three major epitopes of the *env* protein of feline immunodeficiency virus" Molec. Immunol. 29, 565-572 (1992).

216. Fève, B., Baude, B., Krief, S., Strosberg, A.D., Pairault, J. & Emorine, L.J. "Dexamethasone down-regulates β3-adrenergic receptors in 3T3-F442A adipocytes" J. Biol. Chem. 267, 15909-15915. (1992).

217. Strosberg, A.D. "Biotechnologie des récepteurs β-adrénergiques" Path. Biol. 40, 767-772 (1992).

218. Sugasawa, T., Matsuzaki, M., Morooka, S., Foignant, N., Blin, N. & Strosberg, A.D. "*In vitro* study of a novel atypical β-adrenoceptor agonist, SM-11044" Eur. J. Pharmacol. 216, 207-215 (1992).

219. Chouchane, L., Van Spronsen A., Breyer, J., Gugliemi, P. & Strosberg, A.D. "Molecular characterization of a human anti-Rh (D) antibody with a DH segment encoded by a germ-line sequence" Eur. J. Biochem 207, 1115-1121 (1992).

220. Ladenheim, R.G., Lacroix, I., Foignant-Chaverot, N., Strosberg, A.D. & Couraud, P.O. Endothelins stimulate c-fos and NGF expression in astrocytes and astrocytoma. J. Neurochem. 60, 260-266 (1993).

221. Koman, A., Cazaubon, S., Adem, A., Couraud, P.O. & Strosberg, A.D. Different regulatory patterns of M1 and M2 muscarinic receptor subtype RNA in SH-SY5Y human neuroblastoma induced by phorbol ester or DMSO. Neurosci. Lett. 149, 79-82 (1993).

222. Krief, S., Lönnqvist, F., Raimbault, S., Baude, B., Arner, P., Strosberg, A.D., Ricquier, D. & Emorine, L.J. Tissue distribution of b3-adrenergic receptor mRNA in man. J. Clin. Invest. 91 344-349 (1993).

223. Bourdoulous, S., Durieu-Trautmann, O., Strosberg, A.D. & Couraud, P.O. Catecholamines stimulate MHC class I, class II and invariant chain gene expression in brain endothelium through different mechanisms. J. Immunol. 150, 1486-1495 (1993).

224. Durieu-Trautmann, O., Federici, C., Créminion, C., Foignant-Chaverot, N., Roux, F., Claire, M., Strosberg, A.D. & Couraud, P.O. Nitric oxide and endothelin secretion by brain microvessel endothelial cells: regulation by cyclic nucleotides. J. Cell Physiol. 155, 104-111 (1993).

225. Van Spronsen, A., Nahmias, C., Krief, S., Briend-Sutren, M-M., Strosberg, A.D. & Emorine, L.J. The human and mouse β3-adrenergic receptor genes: promoter and intron/exon structure. Eur. J. Biochem 213, 1117-1124 (1993).

226. Van der Zee, E.A., Strosberg, A.D., Bohus, B. & Luiten, P.G.M. Colocalization of muscarinic acetylcholine receptors and protein kinase Cγ in rat parietal cortex. Mol. Brain Res. 18, 152-162 (1993).

227. Van der Zee, De Jong, G.I., E.A., Strosberg, A.D. & Luiten, P.G.M. Muscarinic acetylcholine receptors-expression in astrocytes in the cortex of young and aged rats. GLIA 8, 42-50 (1993).

228. Strosberg, A.D., Camoin, L., Blin, N. & Maigret, B. In receptors coupled to GTP-binding proteins, ligand binding and G-protein activation is a multistep dynamic process. Drug Design & Discovery 9, 199-211(1993).

229. Ravet, V., Blin, N., Guillaume, J-L., Petitjean, F., Cabani_, L. & Strosberg, A.D. High level functional expression of human β1-adrenergic receptor in baculovirus-infected cells screened by a rapid *in situ* procedure. J. of Receptor Res. 13, 541-558 (1993).

230. Holland, E., Ben-Hayyim, G., Faltin, Z., Camoin, L., Strosberg, A.D. & Eshdat, Y. Molecular characterization of salt-stress-associated proteins in citrus: protein and cDNA sequence homology to mammalian gluthione peroxidases. Plant Mol. Biol. 21, 923-927 (1993).

231. Ben-Hayyim, G., Faltin, Z., Gepstein, S., Camoin, L., Strosberg, A.D. & Eshdat, Y. Isolation and characterization of salt-associated proteins in citrus. Plant Sci. 88, 129-140 (1993).

232. Nantel, F., Bonin, H., Emorine, L.J., Zilberfarb, V., Strosberg, A.D., Bouvier, M. & Marullo, S. The human β3-adrenergic receptor is resistant to short-term agonist-promoted desensitization. Mol. Pharmacol. 43, 548-555 (1993).

233. Strosberg, A.D. Structure, function and regulation of adrenergic receptors Protein Sci.12, 1198-1209 (1993).

234. Cazaubon, S., Parker, P.J., Strosberg, A.D. & Couraud, P.O. Endothelins stimulate tyrosine phosphorylation and activity of p42/mitogen-activated protein kinase in astrocytes. Biochem. J. 293, 381-386 (1993).

235. Avrameas, A., Strosberg, Moraillon, Sonigo, P. & Pancino, G-F. Serological diagnosis of feline immunodeficiency Virus (FIV) infection based on synthetic peptides from Env glycoproteins. Res. Virol. 144, 209-218 (1993).

236; Bertin, B., Mansier, P., Makeh, I., Briand, P., Rostene, W., Swynghedauw, B. & Strosberg, A.D. Specific atrial overexpression of G protein-coupled human β1-adrenergic receptors in transgenic mice. Cardiovascular Res. 27, 1606-1612 (1993).

237. Lönnqvist, F., Krief, S., Strosberg, D., Nyberg, B., Emorine, L. & Arner, P. Evidence for a functional β3-adrenergic receptor in man. Br. J. Pharmacol. 110, 929-936 (1993).

228. Emorine, L.J. & Strosberg, A.D. Structure et fonction du récepteur β3-adrénergique. Med/Sci. 9, 1228-1235 (1993).

229. Soula, M., Rothhut, B., Camoin, L., Guillaume, J-L., Strosberg, A.D., Vorherr, T., Burn, P., Meggio, F., Fischer, S. & Fagard, R. Anti-CD3 and phorbol ester induce distinct phosphorylation sites in the SH2 domain of P56LCK. J. Biol. Chem. 268, 27420-27427 (1993).

230. Blin, N., Camoin, L., Maigret, B. & Strosberg, A.D. Structural and conformational features determining selective signal transduction in the β3-adrenergic receptor. Mol. Pharmacol. 44, 1094-1104 (1993).

231. Durieu-Trautmann, O., Bourdoulous, S., Roux, F., Bourre, J.M. Strosberg, A.D. & Couraud, P.O. Immortalized rat brain microvessel endothelial cells: pharmacological characterization. Adv. Exp. Med. Biol. 331, 205-210 (1993).

232. Strosberg, A.D. Molecular biology of β-adrenergic receptors. Neuroprotocols 4, 32-40 (1994).

233. Emorine, L.J., Blin, N. & Strosberg, A.D. The human β3-adrenoceptor: the search for a physiological function. Trends in Pharmacol. Sci. 15, 3-7 (1994).

234. Lazard, D., Villageois, Ph., Briend-Sutren, M., Cavaillé, F., Bottari, S., Strosberg, A.D. & Nahmias, C. Characterization of a membrane glycoprotein having pharmaceutical and biochemical properties of an AT2 angiotensin II receptor from human myometrium. Eur. J. Biochem. 220, 919-926 (1994).

235. Krief, S., F_ve, B., Baude, B., Zilberfarb, V., Strosberg, A.D., Pairault, J. & Emorine, L.J. Transcriptional modulation by n-butyric acid of β1-, β2-, and β3-adrenergic receptor balance in 3T3-F442A adipocytes. J. Biol. Chem. 269, 6664-6670 (1994).

236. Durieu-Trautmann, O., Chaverot-Foignant, N., Cazaubon, S., Strosberg, A.D. & Couraud, P.O. Intercellular adhesion molecule 1 activation induces tyrosine phosphorylation of the cytoskeleton-associated protein cortactin in brain microvessel endothelial cells. J. Biol. Chem. 269, 12536-12540 (1994).

237. Verdot, L., Ferrer-di-Martino, M., Bertin, B., Strosberg, A.D. & Hoebeke, J. Production of anti-peptide antibodies directed against the first and the second extracellular loop of the human serotonin 5-HT1A receptor. Biochimie 76, 165-170 (1994).

238. Nantel, F., Marullo, S., Krief, S., Strosberg, A.D. & Bouvier, M. Cell-specific down-regulation of the β3-adrenergic receptor. J. Biol. Chem. 269, 13148-13155 (1994).

239. Blin, N., Nahmias, C., Drumare, M-F. & Strosberg, A.D. "Mediation of most atypical effects by species homologues of the β3-adrenoceptor. Br. J. Pharmacol. 112, 911-919 (1994).

240. Chochola, J., Yamaguchi, Y., Moguilevski, N., Bollen, A., Strosberg, A.D. & Stanislawski, M. Virucidal Effect of Myeloperoxidase on Human Immunodeficiency Virus Type1-Infected T Cells. Antimicrobial Agents & Chemother. 38, 969-972 (1994).

241. Strosberg, A.D. Functional modulation of β-adrenergic receptors by specific ligands and antibodies. Austral. J. Agricult. Res. chapter 15, 123-127 (1994).

242. Guillaume, J.L., Petitjean, F., Haaseman, M., Bianchi, C., Eshdat, Y. & Strosberg, A.D. Antibodies for the immunochemistry of human β3-adrenergic receptor. Eur. J. Biochem. 224, 761-770 (1994).

243. Blin, N. and Strosberg, A.D. "The human β3-adrenoceptor mediates lipid metabolism : other physiological roles remain to be determined. Trends in Pharmacol. Sci. 15, 281-282 (1994).

244. Bertin, B., Freissmuth, M., Jockers, R., Strosberg, A.D. & Marullo, S. Cellular signaling by an agonist-activated receptor/Gsa fusion protein. Proc. Natl. Acad. Sci. USA 91, 8827-8831 (1994).

245. Dolan, J.A., Muenkel, H.A., Burns, M.G., Pellegrino, S.M., Fraser, C.M., Pietri, F., Strosberg, A.D., Largis, E.E., Dutia, M.D., Bloom, J.D., Bass,A. S., Tanikella, T.K., Cobuzzi, A., Lai, F.M. & Claus, T.H. Beta-3 adrenoceptor selectivity of the dioxolane dicarboxylate phenethanolamines. J. Pharmacol. Exp. Therap. 269, 1000-1006 (1994).

246. Jockers, R., Linder, M., Hohenegger, M., Nanoff, C., Bertin, B., Strosberg, A.D., Marullo, S. & Freissmuth, M. .Species differences in the G protein selectivity of the human and bovine A1-adenosine receptor. J. Biol. Chem. 269, 32077-32084 (1994).

247. Cazaubon, S., Ramos-Morales, F., Fischer, S., Schweighoffer, F., Strosberg, A.D. & Couraud, P.O. Endothelin induces phosphorylation and GRB2 association of SHC in astrocytes. J. Biol. Chem. 269, 24805-24809 (1994).

248. Lazard, D., Briend-Sutren, M.M., Villageois, P.Mattei, M.G., Strosberg, A.D. & Nahmias, C. Molecular characterization and chromosome localization of a human angiotensin II AT2 receptor gene highly expressed in fetal tissues. Receptors & Channels 2, 271-280 (1994).

249. Marullo, S., Nantel, F., Strosberg, A.D. & Bouvier, M. Variability in the regulation of β-adrenoceptor subtypes. Biochem. Soc. Trans. 23, 126-129 (1995).

250. Nahmias, C., Cazaubon, S., Briend-Sutren, M.M., Lazard, D., Villageois, P. & Strosberg, A.D. Angiotensin II AT2 receptors are functionally coupled to protein tyrosine dephosphorylation in N1E-115 neuroblastoma cells. Biochem. J. 306, 87-92 (1995).

251. Nantel, F., Bouvier, M., Strosberg, A.D. & Marullo, S. Functional effects of long-term activation on human β_2- and β_3-adrenoceptor signalling. Br. J. Pharmacol. 114, 1045-1051 (1995).

252. Sibille, P., Avrameas, A., Moraillon, A. Richardson, J., Sonigo, P., Pancino, G. & Strosberg, A.D. Comparison of serological tests for the diagnosis of Feline Immunodeficiency Virus infection of cats. Vet. Microbiol. 45, 259-267 (1995).

253. Fédérici, C., Camoin, L., Cr_minion, C., Chaverot, N., Strosberg, A.D. & Couraud, P.O. Cultured astrocytes release a factor that decreases endothelin-1 secretion by brain microvessel endothelial cells. J. Neurochem. 64, 1008-1015 (1995).

254. Blin, N., F_d_rici, C., Koscielniak, T. & Strosberg, A.D. Predictive quantitative structure-activity (QSAR) analysis of β_3-adrenergic ligands. Drug Design and Discovery 12, 297-311 (1995).

255. Verdot, L., Bertin, B., Guillotteau, D., Strosberg, A.D. & Hoebeke, J. Characterization of pharmacologically active anti-peptide antibodies directed against the first and the second extracellular loops of the serotonin 5HT1A receptor. J. Neurochem. 65, 319-328 (1995).

256. Bourdoulous, S., Bensaid, A., Martinez, D., Sheikboudou, C., Trap, I., Strosberg, A.D. & Couraud, P.O. 1995. Infection of bovine brain microvessel endothelial cells with *Cowdria Ruminantium* elicits interleukin-1b, -6 and -8 mRNA production and expression of an unusual MHC classe DQa transcript. J. Immunol. 154, 4032-4038 (1995).

257. Pietri-Rouxel, F. & Strosberg, A.D. Pharmacological characteristics and species-related variations of β3-adrenergic receptors. Fundam. Clin. Pharmacol. 9, 211-218 (1995).

258. Fève, B., Piétri-Rouxel, F., El Hadri, K., Drumare, M.F. & Strosberg, A.D. Long-term phorbol ester treatment down-regulates the β3-adrenergic receptor in 3TF442A adipocytes. J. Biol. Chem. 270, 10952-10959 (1995).

259. Bourdoulous, S., B_raud, E., Lepage, C., Zamora, A.J., Ferry, A., Bernard, D., Strosberg, A.D. & Couraud, P.O. Anergy induction in encephalitogenic T cells by brain microvessel endothelial cells is ihhibited by interleukin-1. Eur. J. Immunol. 25, 1176-1183 (1995).

260. Nahmias, C. & Strosberg, A.D. The Angiotensin II AT2 receptor: searching for signal transduction and physiological function. Tr. Pharmacol. Sci. 16, 223-225 (1995).

261. Piétri-Rouxel, F., Lenzen, G., Kapoor, A., Drumare, M.F., Archimbault, P., Strosberg, A.D. & Manning, B. Molecular cloning and pharmacological characterization of the bovine β3 adrenergic receptor. Eur. J. Biochem. 230, 350-358 (1995).

262. Roulot, D., Durand, H., Coste, T., Rautureau, J., Strosberg, A.D., Benarous, R. & Marullo, S. Quantitative analysis of TGFb1 mRNA in the liver of patients with chronic hepatitis C: absence of correlation between high levels and severity of disease. Hepatology 21, 298-304 (1995).

263. Clément, K., Vaisse, C., Manning, B., Basdevant, A., Guy-Grand, B., Shuldiner, A., Froguel, P. & Strosberg, A.D. Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. New Engl. J. Med. 333, 352-354 (1995).

264. Walston, J., Silver, K., Bogardus, C., Knowler, W.C., Celi, F. S., Austin, S., Manning, B., Strosberg, A.D., Stern, M.P., Raben, N., Sorkin, J.D., Roth, J. & Shuldiner, A.R. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3-adrenergic receptor gene. New Engl. J. Med. 333, 343-347 (1995).

265. Strosberg, A.D. A lean treatment of a 'fat' subject" on "β3-adrenergic agonism: a new concept in human pharmacology. by D.E. Golberg & W.H. Frishman, Tr. Pharmacol. Sci. 16, 175-176 (1995).

266. Piétri-Rouxel, F., Drumare, M.F. & Strosberg, A.D. Pharmacological characteristics of β3-adrenergic receptor. Pharmacology Communications 6, 229-236 (1995).

267. Strosberg, A.D. Structure and function of the β3-adrenoceptor. Int. J. Obes. 18, S2-pp40 (1995).

268. Méjean-Galzi, A., Guillaume, J.L. & Strosberg, A.D. Carazolol: a potent, selective β3-adrenoceptor agonist. Eur. J. Pharmacol. 291, 359-366 (1995).

269. Strosberg, A.D. Structure, Function and Regulation of the three β-adrenergic receptors. Obesity Research 3, 501S-505S (1995).

270. Strosberg, A.D. & Manning, B. St J. Le récepteur β3-adrenergique : un gène de poids. Med/Sci. 11, 1460-1462 (10, Octobre 1995).

271. Lazarini, F., Strosberg, A.D., Couraud, P.O. & Cazaubon, S.M. Coupling of ETB endothelin receptor to map kinase activation and DNA synthesis in primary culture of rat astrocytes. J. Neurochem. 66, 459-465 (1996).

272. Jockers, R., DaSilva, A., Strosberg, A.D., Bouvier, M. & Marullo, S. New molecular and structural determinants involved in β_2-adrenergic receptor uncoupling and sequestration; delineation using chimeric β_3/β_2-adrenergic receptors. J. Biol. Chem. 271, 9355-9362 (1996).

273. Strosberg, A.D. Rôle du récepteur adrénergique dans le métabolisme des lipides. Oléa. Corps Gras Lipides, OCL 3, 83-85 (1996).

274. Laisney, I., Benjamin, H., Gefter, M. & Strosberg, A.D. Permissive residues within the minimal epitopes of neutralizing monoclonal antibodies to the V3 loop of HIV-1. Eur. J. Immunol. 26, 1634-1640 (1996).

275. Koman, A., Cazaubon, S., Couraud, P.O., Ullrich, A. & Strosberg, A.D. Molecular characterization and in vitro biological activity of placentin. A new member of the insulin gene family. J. Biol. Chem. 271, 20238-20241 (1996).

276. Strosberg, A.D & Pietri-Rouxel, F. Function and regulation of the β_3-adrenoceptor. Tr. in Pharmacol. Sci. 17, 373-381 (1996).

277. Strosberg, A.D. Du gène au médicament: la découverte du récepteur β3-adrénergique par la pharmacologie inverse. Med/Sci 12, 17-20 (Octobre 1996 n° special)

278. Strosberg, A.D. G-protein coupled"R7G" receptors. Cancer Surveys , Cell Signalling: 27, 65-83 (1996).

279. Mansier, P., Médigue, C., Charlotte, N., Vermeiren, C., Coraboeuf, E., Deroubai, E., Chevalier, B., Clairambault, J., Carré, F., Dahkli, T., Bertin, B., Briand, P., Strosberg, A.D. & Swynghedauw, B. Decreased heart rate variability in transgenic mice overexpressing atrial β1-adrenoceptors. Am. J. Physiol. 271, H1465-H1472 (1996).

280. Strosberg, A.D. & Froguel, Ph. β3-adrenoceptor gene variant in obesity and insulin resistance. The Lancet 348, 1585 (1996).

281. Charon, C., Krief, S., Diot-Duput, F., Strosberg, A.D., Emorine, L.J. & Bazin, R. Early alterations in the brown adipose tissue adenylate cyclase system of preobese Zucker fa/fa pups: decreased Gs and β3-adrenoceptor activities. Biochem. J. 312, 781-788 (1996).

282. Federici, C., Camoin, L., Hattab, M., Strosberg, A.D. & Couraud, P.O. Association of the cytoplasmic domain of intracellular adhesion molecule-1 with glyceraldehyde-3-phosphate dehydrogenase and b tubulin. Eur. J. Biochem. 238, 173-180 (1996).

283. Bengtsson, T., Redegren, K., Strosberg, A.D., Nedergaard, J. & Cannon, B. Down-regulation of β3-adrenoceptor gene expression in brown-fat cells is transient and recovery is dependent upon a short-lived protein factor. J. Biol. Chem. 271, 33366-33375 (1996).

284. Strosberg, A.D. & Pi_tri-Rouxel, F. "The β3-adrenoceptor constitutes indeed a versatile receptor" Tr. in Pharmacol. Sci. 18, 52 (1997).

285. Quinonero, J., Tchélingérian J.L., Vignais, L., Foignant-Chaverot, N., Colin, C., Horellou, P., Liblau, R., Barbin, G., Strosberg, A.D., Jacque, C. & Couraud, P.O. "Gene transfer to the central nervous system by transplantation of cerebral endothelial cells". Gene Therapy 4, 111-119 (1997).

286. Piétri-Rouxel, F. & Strosberg, A.D."Le récepteur β3-adrénergique humain: le poids des faits" Médecine/Sci. 13, 283-284 (1997).

287. Strosberg, A.D. "Structure and function of the b3-adrenergic receptor"Ann. Rev. Pharmacol & Toxicol. 37, 421-450 (1997).

288. Zilberfarb, V., Piétri-Rouxel, F., Jockers, R., Krief, S., Delouis, C., Issad, T. & Strosberg, A.D. "Human immortalized brown adipocytes express functional β3-adrenoceptor coupled to lipolysis" J. Cell Sci. 110, 801-807 (1997).

289. Sibille, P., Ternynck, T., Nato, F., Buttin, G., Strosberg, A.D. & Avrameas, A. "Mimotopes recognized by polyreactive anti-DNA antibodies" Eur. J. Immunol. 27, 1221-1228 (1997).

290. Conway, S., Drew, J.E., Canning, S.J., Barrett, P., Jockers, R., Strosberg, A.D., Guardiola-Lemaitre, B., Delagrange, P. & Morgan P.J. "Identification of Mel 1a melatonin receptors in the human embryonic kidney cell line HEK293: evidence of G protein-coupled receptors which do not mediate the inhibition of stimulated cyclicAMP levels" FEBS Lett. 407, 121-126 (1997).

291. Gotoda, T., Manning, B., Goldstone, A.P., Imrie, H., Evans, A.L., Strosberg, A.D., McKeigue, P.M., Scott, J. & Aitman T.J. "Leptin receptor gene variation and obesity: lack of association in a white British male population" Hum. Mol. Genetics 6, 869-876 (1997).

292. Strosberg, A.D. "Structure and function of the β3-adrenoreceptor " Adv. in Pharmacol. 42, 511-513 (1997).

293. Bertin, B., Strosberg, A.D. & Marullo, S. "The human b2-adrenergic receptor/Gsa fusion protein expressed in two ras-dependent murine carcinoma cell lines prevents tumor growth in syngeneic mice" Int. J. Cancer 71, 1029-1034 (1997).

294. Bedecs, K., Elbaz, N., Sutren, M., Masson, M., Susini, C., Strosberg, A.D. & Nahmias, C. "Angiotensin AT2 receptors inhibit epidermal growth factor-induced mitogen-activated protein kinase cascade and stimulate the catalytic activity of protein tyrosine phosphatase SHP-1" Biochem. J. 325, 449-454 (1997).

295. Jockers, R., Petit, L., Lacroix, I., de Coppet, P., Barrett, P., Morgan, P.J., Guardiola, B., Delagrange, P., Marullo, S. & Strosberg, A.D. "Novel isoforms of Mel1c melatonin receptors modulating intracellular cGMP levels" Mol. Endocrinol. 11, 1070-1081 (1997).

296. Bailleul, B., Akerblom, I. & Strosberg, A.D. "The leptin receptor promoter controls the expression of a second distinct leptin receptor gene-related protein" Nucl. Acid Res. 25, 2752-2758 (1997).

297. Sugasawa, T., Matsuzaki- Fujita, M., Guillaume, J.L., Camoin, L., Morooka, S. & Strosberg, A.D. "Characterization of a novel 34kDa SM-11044 binding protein, which may mediate relaxation of depolarized rat colon tonus" J. Biol. Chem. 272 , 21244-21252 (1997).

298. Barrett, P., Conway, S., Jockers, R., Strosberg, A.D., Guardiola, B., Delagrange, P. & Morgan P.J. "Cloning and expression of a polymorphic variant of the ovine Mel 1a melatonin receptor" Biochem. Biophys. Acta 1356, 299-307 (1997).

299. Cazaubon, S., Chaverot, N., Romero, I.A., Girault, J.A., Adamson, P., Strosberg, A.D. & Couraud, P.O. "Growth factor activity of endothelin-1 in primary astrocytes mediated by adhesion-dependent and independent pathways" J. Neurosci. 17, 6203-6212 (1997).

300. Bertin, B., Jockers, R., Strosberg, A.D. & Marullo, S. "Activation of a β2-adrenergic receptor/Gsα fusion protein elicits a desensitization-resistant cAMP signal capable of inhibiting proliferation of two cancer cell lines" Receptors & Channels 5, 41-51 (1997).

301. Piétri-Rouxel, F., Manning, B., Gros, J. & Strosberg, A.D. "The biochemical effect of the naturally occuring Trp64Arg mutation on human β3-adrenoceptor activity" Eur. J. Biochem. 247, 1174-1179 (1997).

302. Zhang, J.M., Dix, J., Langtimm-Sedlack, C.J., Trusk, T., Schroeder, B., Hoffmann, R., Strosberg, A.D., Winslow, J.W. & Sieber-Blum, M. "Neurotrophin-3-and norepinephrine-mediated adrenergic differentiation and the inhibitory action of desipramine and cocaine" J. Neurobiol. 32, 262-280 (1997).

303. Loisel, T.P., Ansanay, H., St-Onge, S., Gay, B., Boulanger, P., Strosberg, A.D., Marullo, S. & Bouvier, M. "Recovery of homogeneous and functional β2-adrenergic receptors from extracellular baculovirus particles" Nature Biotech. 15, 1300-1304 (1997).

304. Strosberg, A.D. "Towards the development and use of human-selective agonists for the pharmacologic treatment of obesity and diabetes" J. Endocrinol. 155, 221-222 (1997).

305. Clément, K., Manning, B., Basdevant, A., Strosberg, A.D., Guy-Grand, B. & Froguel, P. "Gender effect of the Trp64Arg mutation in the β3-adrenergic receptor gene on weigth gain in morbid obesity" Diabetes & Metabolism 23, 424-427 (1997).

306. Sibille, P. & Strosberg, A.D. "The epitope of a monoclonal anti-FIV antibody defined by screening a phage peptide library" Immunol. Lett. 59,133-137 (1997).

307. Strosberg, A.D. "Association of β3-adrenoreceptor polymorphism with obesity and diabetes: current status" Tr. in Pharmacol. Sci. 18, 449-454 (1997).

308. Romero, I.A., Texeira, A., Strosberg, A.D., Cazaubon, S. & Couraud, P.O. "The HIV-1 nef protein inhibits extracellular signal regulated kinase-dependent DNA synthesis in a human astrocytic cell line" J. Neurochem. 70, 778-785 (1998).

309. Gros, J., Manning, B., Pi_tri-Rouxel, F., Guillaume, J.L., Drumare, M.F. & Strosberg, A.D. "Site-directed mutagenesis of the human beta-3 adrenoceptor: transmembrane residues involved in ligand binding and signal transduction" Eur. J. Biochem. 251, 590-596 (1998).

310. Bernardin G., Strosberg A.D., Bernard A., Mattei M. & Marullo S. "Beta-adrenergic receptor-dependent and -independent stimulation ofadenylate cyclase is impaired during severe sepsis in humans. Intensive Care Med. 24, 1315-1322 (1998).

311. Lenzen, G., Pietri-Rouxel, F., Drumare, M.F., Amiard, A., Guillot, S., Archimbault, P. & Strosberg, A.D. "Genomic cloning and species-specific properties of the recombinant canine β3-adrenoceptor" Eur. J. Pharmacol. 363, 217-227 (1998).

312. Petit, L., Guardiola, B., Delagrange, P., Jockers, R. & Strosberg, A.D. "Signalisation des récepteurs de la mélatonine" Thérapie 53, 421-428 (1998).

313. Ito, M., Grujic, D., Abel, E.D., Vidal-Puig, A., Susulic, V.S., Lawitts, J., Strosberg, A.D. & Lowell, B.B. "Mice expressing human but not murine β3-adrenergic receptors under the control of human gene regulatory elements" Diabetes 47, 1464-1471 (1998).

314. Strosberg, A.D. "Les signaux cellulaires passés au crible" Biofutur 184, 45-47 (1998).

315. Etienne, S., Adamson, P., Greenwood, J., Strosberg, A.D. Cazaubon, S. & Couraud, P.O. "ICAM-1 signalling pathways associated with Rho activation in microvascular brain endothelial cells" J. Immunol. 161, 5755-5761 (1998).

316. Issad, T., Strobel, A., Camoin, L., Ozata, M. & Strosberg, A.D. "Leptine et puberté dans l'espèce humaine : l'hypothèse de la masse adipeuse critique réactualisée " Diabetes & Metabolism 24, 376-378 (1998).

317. Strobel, A., Combettes-Souverain, M., Doaré, L., Strosberg, A.D. & Issad, T. "Rat Uncoupling Protein 2 (UCP2): Expression in obese ventromedial hypothalamus (VMH)-lesioned animals" Int. J. Obes. 22, 1121-1126 (1998).

318. Piétri-Rouxel, F. & Strosberg, A.D. "Les récepteurs β-adrénergiques et le tissu adipeux" Reprod. Hum. & Hormones 11, 493-497 (1998).

319. Jockers, R., Petit, L., Brydon, L., de Coppet, P. & Strosberg, A.D. "Structure et fonction des récepteurs de la mélatonine" C.R. Soc. Biol. 192, 659-667 (1998).

320. Strosberg, A.D., Gerhardt, C., Gros, J., Jockers, R. & Piétri-Rouxel, F. "On the putative existence of a fourth β-adrenoceptor: proof is still missing" Tr. in Pharmacol. Sci. 19, 165-166 (1998).

321. Jockers, R., Issad, T., Zilberfarb, V., de Coppet, P., Marullo, S. & Strosberg, A.D. "Desensitization of the β-adrenergic response in human brown adipocytes" Endocrinology 136, 2676-2684 (1998).

322. Anthony, A., Schepelmann, S., Guillaume, J.L., Strosberg, A.D., Dhillon, A.P., Pounder, R.E. & Wakefield, A.J. "Localization of the beta3-adrenoceptor in the human gastrointestinal tract: an immuno-histochemical study" Alimentary & Pharmacol. Ther. 12, 519-525 (1998).

323. Issad, T., Strobel, A., Camoin, L., Ozata, M. & Strosberg, A.D. "La leptine : un signal pour le déclenchement de la puberté dans l'espèce humaine" Med/Sci 14, 349-351 (1998).

324. Strobel, A., Issad, T., Camoin, L., Ozata, M. & Strosberg, A.D. "A leptin missense mutation associated with hypogonadism and morbid obesity" Nature Genetics 18, 213-215 (1998).

325. Roka, F., Brydon, L., Waldhoer, M., Strosberg, A.D., Freissmuth, M., Jockers, R. & Nanoff, C. "Tight association of the human Mel1a-melatonin receptor and G_i: precoupling and constitutive activity" Mol. Pharmacol. 56, 1014-1024 (1999).

326. Gerhardt, C.C., Gros, J., Strosberg, A.D. & Issad, T. "Stimulation of ERK1/2 pathway by the human β3-adrenergic receptor: new pharmacological profile and mechanism of activation" Mol. Pharmacol. 55, 255-262 (1999).

327. Strosberg, A.D. & Issad, T. "The role of mutations in leptin and leptin receptors' genes in human obesity and reproductive function" Tr. in Pharmacol. Sci. 20, 227-230 (1999).

328. Luyckx, F.H., Scheen, A.J., Strosberg, A.D., Gielen, J.E. & Lefèbvre, P.J. "Influence of the A Ø G (-3826) uncoupling protein-1 gene (UCP1) variant on the dynamics of body weight before and after gastroplasty in morbidly obese subjects" Int. J. Obesity Relat. Metab. Disord. 22, 1244-1245 (1999).

329. Faltin, Z., Camoin, L., Ben-Hayyim, G., Perl, A., Beeor-Tzahar, T., Strosberg, A.D., Holland, D. Eshdat, Y. Cysteine is the presumed catalytic residue of Citrus sinensis phospholipid hydroperoxide glutathione peroxidase over-expressed under salt stress. Physiol. Plant 104, 741-746 (1999).

330. Etienne, S., Bourdoulous, S., Strosberg, A.D. & Couraud, P.O. "MHC class II engagement in brain endothelial cells induces protein kinases IL-6secretion and phosphorylation of cAMP response element-binding protein" J. Immunol. 163, 3636-3641 (1999).

331. Strobel, A., Siquier, K., Zilberfarb, V., Strosberg, A.D. & Issad, T. "Effect of thiazolidinediones on UCP2 expression in human PAZ-6 adipocytes" Diabetologia 42, 527-533 (1999).

332. Federici, C., Eshdat, Y., Richard, I., Bertin, B., Guillaume, J.L., Hattab, M., Beckmann, J., Strosberg, A.D. & Camoin, L. "Purification and identification of two putative autolytic sites in human calpain 3 (p94) expressed in heterologous systems" Arch. Biochem. & Biophysics 363, 237-245 (1999).

333. Roulot, D., Sevcsik, A.M., Coste, T., Strosberg, A.D. & Marullo, S. "Role of transforming growth factor β type II receptor in hepatic fibrosis: studies of humanchronic hepatitis C and experimental fibrosis in rat" Hepatology 29, 1730-1738 (1999).

334. Laisney, I.L. & Strosberg, A.D. "Dual specificity of a human neutralizing monoclonal antibody, specific for the V3 loop of gp120 (HIV-1)" Immunology Lett. 67, 185-192 (1999).

335. Petit, L., Lacroix, I., de Coppet, P., Strosberg, A.D. & Jockers, R. "Differential signaling of human Mel1a and Mel1b melatonin receptors through the cyclic Guanosine 3'-5' monophosphate pathway" Biochem. Pharmacol. 58, 633-639 (1999).

336. Jockers, R., Angers, S., Da Silva, A., Benaroch, P., Strosberg, A.D., Bouvier, M. & Marullo, S. "β_2-adrenergic receptors down-regulation: evidence for a pathway that does not require endocytosis" J. Biol. Chem. 274, 28900-28908 (1999).

337. Gros, J., Gerhardt, C. & Strosberg, A.D. "Expression of human beta3-adrenergic receptor induces adipocyte-like features in CHO/K1 fibroblasts" J. Cell Sci. 112, 3791-3797 (1999).

338. Brydon, L., Roka, F., Petit, L., de Coppet, P., Tissot, M., Barrett, P., Morgan, P.J., Nanoff, C., Strosberg, A.D. & Jockers, R. " Dual signalling of human Mel1a Melatonin receptors via Gi2, Gi3 and Gq/11 proteins" Mol. Endocrinol. 13, 2025-2038 (1999).

339. Proenza, A.M., Poissonnet, C.M., Ozata, M., Ozen, S., Guran, S., Palou, A. & Strosberg, A.D. Association of sets of alleles of genes encoding ©3-adrenoceptor, uncoupling protein 1 and lipoprotein lipase with increased risk of metabolic complications in obesity. Int. J. Obesity 1, 93-100 (2000).

340. Texeira, A., Chaverot, N., Strosberg, A.D. & Cazaubon, S. "Differential regulation of cyclin D1and D3 expression in the control of astrocyte proliferation induced by endothelin-1" J. Neurochem. 74, 1034-1040 (2000).

341. Elbaz, N., Bedecs, K., Masson, M., Sutren, M., Strosberg, A.D. & Nahmias, C. "Functional transactivation of insulin receptor by growth inhibitory angiotensin II AT2 receptor" Mol. Endocrinol.14(6) 795-804 (2000).

342. Mercer, J., Moar, K.M., Hoggard, N., Strosberg, A.D., Froguel, P. & Bailleul, B. "B219/OB-R 5'UTR and leptin receptor gene-related protein (OB-RGRP) gene expression in mouse brain and placenta: evidence for tissue-specific leptin receptor promoter activity " J. of Neuroendocrinology 12, 649-655 (2000).

343. Hazebrouck, S., Camoin, L., Faltin, Z., Strosberg, .D. & Eshdat, Y. "Enhanced Activity of an Engineered Selenocysteine-Containing Analog of a Plant Phospholipid Hydroperoxide Glutathione Peroxidase Expressed in *E. coli*" J. Biol. Chem. 275, 28715-28721 (2000).

344. Anthony, A., Sim, R. Guillaume, J.L., Strosberg, A.D., Dhillon, A.P., Pounder, R.E. & Wakefield, A.J. "beta3-adrenergic receptors in human pancreatic islet and duodenal somastatin neuroendocrine cells" Aliment Pharmacol. Ther. 14, 579-585 (2000).

345. Esterbauer, H., Oberkofler, H., Krempler, F., Strosberg, A.D. & Patsch, W. "The uncoupling protein-3 (UCP3) gene is transcribed from tissue-specific Promoters in humans, but not in rodents" J. Biol. Chem. 275, 36394-36399 (2000).

346. Jockers, R. & Strosberg, A.D. "Expression of beta-adrenergic receptors in *E. coli*" in Methods Mol. Biol. 126, 215-220 (2000).

347. Strosberg, A.D. & Guillaume, J.L. "Expression of beta-adrenergic receptors in recombinant baculovirus-infected insect cells" in Methods Mol. Biol. 126, 207-214 (2000).

348. Strosberg A.D. "Functional Proteomics to Exploit Genome Sequences". Cell. Mol. Biology, 47(8), 1295-1299 (2000)

349. Zilberfarb, V., Siquier, K., Strosberg, A.D. & Issad, T. "Effect of dexamethasone on adipocyte differentiation markers and tumour necrosis factor alpha expression in human PAZ6 cells" Diabetologia 44, 377-386 (2001).

350. Gerhardt, C.C., Romero, I.A., Cancello, R., Camoin, L. & Strosberg, A.D. "Chemokines control fat accumulation and leptin secretion by cultured human adipocytes" Mol. & Cell. Endocrinology 175, 81-92 (2001).

351. Sugasawa, T., Lenzen, G., Simon, S., Hidaka, J., Cahen, A., Guillaume, J.L., Camoin, L., Nahmias, C. & Strosberg, A.D. "SM-11044 binding protein (SMBP) belongs to an emerging family of EMP70-related multispanning membrane proteins" Gene 273, 227-237 (2001).

352. Strosberg, A.D. "Functional proteomics to exploit genome sequences" Cell Mol. Biol. 47, 1295-1299 (2001).

353. Brydon L., Petit L., Delagrange P., Strosberg , A.D. & Jockers R. "Functional expression of MT2 (Mel1b) melatonin receptors in human PAZ6 adipocytes" Endocrinology 142, 4264-4271 (2001).

354. Esterbauer, H., Schneitler, C., Oberkofler, H., Ebenbichler, C., Paulweber, B., Sandhofer, F., Ladurner, G., Hell, E., Strosberg, A.D., Patsch,J.R., Krempler, F. & Patsch, W. "A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans" Nat. Genet. 28, 178-183 (2001)

355. Chouchane L., Danguir, J., Beji, C., Bouassida, K., Camoin, L., Sfar, H., Gabbouj, S. & Strosberg, A.D. "Genetic variation in the stress protein hsp70-2 gene is highly associated with obesity" Int. J. Obes. Relat. Metab. Disord. 25, 462-466 (2001).

356. Legrain P., Strosberg D. "Protein interaction domain mapping for the selection of validated targets and lead compounds in the anti-infectious area". Current Pharmaceutical Design, 8, 1189-1198 (2001).

357. Strosberg A.D. "Where is Proteomics Going?" Current Drug Discovery, May 2002

358. Soll D., Strosberg A.D. "Techniques Genome-Wide Technologies: The First Half of the Story". Cur. Op. in Microbiology 5, 311-312 (2002).

359. Strosberg A.D. "Protein Interaction Mapping for Target Validation: the Need for an Integrated Combinatory Process Involving Complementary Approaches". Cur Op. in Mol. Therapeutics, 4 (6), 594-600 (2002).

360. Hazan U., Romero, I.A., Cancello, R., Valente, S., Perrin, V., Mariot, V., Dumonceaux, J., Gerhardt, C.C., Strosberg, A.D., Couraud, P.O. & Pietri-Rouxel, F. "Human adipose cells express CD4, CXXR4, CCR5 and receptors : a new target cell type for the immunodeficiency virus-1 ? " FASEB J. 16, 1254-1256 (2002).

361. Oberkofler, H., Esterbauer, H., Linnemayr, V., Strosberg, A.D., Krempler, F. & Patsch, W. "Peroxisome proliferator-activated receptor (PPAR) gamma coactivator-1 recruitment regulates PPAR subtype specificity" J. Biol. Chem. 277, 16750-16757 (2002).

362. El Hadri, A., Nicollé, E., Guillaume, M.C., Leclerc, G., Piétri-Rouxel, F., Strosberg, A.D. & Archimbault, P. "Synthesis of beta3-adrenergic agonistic activities of a novel series of aryloxypropamines" Die Pharmazie 56, 517-522 (2002).

363. Strosberg A.D. "De la cible génétique au médicament : le point de vue d'un entrepreneur de biotechnologie" dans le Génome : avancées scientifiques (2003).

364. Strosberg A.D., Daviet L., Borg-Capra C. "Functional Proteomics Applied to Obesity Research" in "Progress in Obesity Research", Vol.9, Chapter 48, pp 229-233, G. Medeiros-Neto, A. Halpern and C. Bouchard Eds, John Libbey Eurotext Ltd (2003).

CONTRIBUTION TO BOOKS 1970 - 2003

Professor A.D. STROSBERG

1. Strosberg, A.D. "Etude immunochimique du lysozyme de blanc d'oeuf de poule". Thèse de doctorat. Université Libre de Bruxelles (1970).

2. Haber, E and Strosberg, A.D. "Structure and function of homogeneous antibodies, in specific receptors of antibodies, antigens and cells". 3rd Internat. Convoc. of Immunology, Buffalo, New York, Ed. by D. Pressman, T.B. Tomasi, Jr., A.L. Grossberg and H.R. Rose, p. 236-258, Karger (1973).

3. Strosberg, A.D. "Inherited control mecanisms in mammalian gene expression". In "Regulation of the Immune system", Academic Press, New York 115-122 (1977).

4. Guellaen, G., Yates-Aggerbeck, M., Vauquelin, G., Strosberg, A.D. and Hanoune, J. "Characterization with 9, 10 [^{3}H] dihydroergocryptine of the α-adrenergic receptor of the hepatic plasma membrane : comparison with the β-adrenergic receptor in normal and adrenalectomized rats". Colloques INSERM (1977).

5. Grosswasser, J., Hoebeke, J., Vamos, E. and Strosberg, A.D. "Kinetic analysis of purified human placental α-L-Fucosidase by a continuous fluorimetric assay. (Communication) VIIIth International Symposium on Clinical Enzymology, Venice, Italy (1978).

6. Strosberg, A.D., Marescau, B., Thielemans, K., Vray, B., Karcher, D., and Lowenthal, A. "Cross-idiotypic specificity among immunoglobulins in subacute sclerosing panencephalitis and multiple sclerosis". In : "Humoral Immunity in Neurological Diseases" Eds.: D. Karcher, A. Lowenthal, and A.D. Strosberg, Plenum Pub. Corp., Antwerp, p. 97-103 (1979).

7 Strosberg, A.D., Bollengier, F., Mahler-Rabinovitch, N., Valdimarsson, H., and Lowenthal, A. "Structural studies of immunoglobulins from patients affected by subacute sclerosing panencephalitis". In "Humoral Immunity in Neurological Diseases". Eds: D. Karcher, A. Lowenthal, and A.D. Strosberg, Plenum Pub. Corp., Antwerp, p. 319-325 (1979).

8a Foriers, A., Baumann, C., Lieber, S., de Neve, R. and Strosberg, A.D. "Structure-function relationship among leguminous plant lectins". In "Protides of the Biological Fluids XXVII". Ed. H. Peeters, Pergamon Press, Headington Hill Hall, Oxford, England (1979).

8b Hoebeke, J., Vray, B., Foriers, A. and Strosberg, A.D. "Redistribution of lentil lectin receptors on rabbit polymorphonuclear membranes". In "Mode of Action of Lectins" "Protides of Biological Fluids 27th Colloquium, Ed. H. Peeters, Pergamon Press, Headington Hill Hall, Oxford, England (1979).

9 Congress of Immunol., Paris 1980. Ed. by J. Dausset and M. Fougereau (1980).

10 Vray, B., Saint-Guillain, M., Hoebeke, J., Leloup, R. and Strosberg, A.D. "Kinetic and morphological studies of phagocytosis" In "Triggering of Phagocytic Cells". Ed. by M.P. Dierich, G. Fust and M.W. Turner, Medicina Public., Budapest, p. 169-173 (1980).

11 Lauwereys, M., Dognin, M.J., Emorine, L., Ayadi, H. and Strosberg, A.D. "Multiple amino acid differences between products of allelic constant region genes: Clues for evolution?" ICN-UCLA Symposium on Immunoglobulin Idiotypes. Eds. Janeway, C., Sercarz, E.E. and Wigzell, H., Academic Press, p.209-217 (1981).

12 Bauman, C.M., Rudiger, H. and Strosberg, A.D. "Vicia cracca lectins and their evolution". In "Lectins, Biology and Biochemistry". Ed. by Bog-Hansen, T.C., (W. de Gruyter, Berlin), Vol. 1, 93-100 (1981).

13. Shannon, L., Hankins, M. and Strosberg, A.D. "The α- and ß-subunits of pea lectin are the result of a post-translational cleavage of a precursor chain". In "Lectins, Biology and Biochemistry", Ed. by Bog-Hansen, T.C., (W. de Gruyter, Berlin), Vol. 2, 81-93 (1982).

14. Lauwereys, M., Van Driessche In "Lectins, Biology and Biochemistry", Ed. by Bog-Hansen, T.C., De Gruyter, W. (Berlin), Vol. 3 (1982).

15. Guillet, J.G., Marche, P., Hoebeke, J. and Strosberg, A.D. "Binding characteristics of a monoclonal antibody against rabbit thymocytes". Proc. 29th Colloquium Prot. Biol. Fluids. Ed. Peeters, H., Pergamon Press, (1982).

B Schmutz, A. and Strosberg, A.D. "Immunochemical studies of the β-adrenergic catecholamine receptors: a two-way approach". Proc. 29th Colloquium Prot. Biol. Fluids. Ed. Peeters, H., Pergamon Press, p. 493-496 (1982).

17. Strosberg, A.D. "An idiotype anti-idiotype network involving the β-adrenergic hormone receptor". In "Idiotype: Antigens on the Inside". Eds. Berek, C., Ettinger, H. and Julius, M., Editions Roche, Basel, p.116-120 (1982)

18. Strosberg, A.D. "Biochemical and immunochemical studies of the β-adrenergic receptor". In "Cell Regulation by Intracellular Signals". Eds, Swillens, S., Dumont, J.E., Plenum Pub. Corp. p.87-94 (1982).

19. Paroutaud, P., Dutka, S., Haimovich, J. and Strosberg, A.D. "Analyse de la structure des immunoglobulines par chromatographie à haute performance en phase liquide" in Ed. des Colloques de l'INSERM 115, 161-168 (1983).

20. Strosberg, A.D., Lauwereys, M. and Foriers, A. "Molecular evolution of legume lectins". In "Chemical Taxonomy, Molecular Biology and Function of Plants". Etzler, E.M., Alan Liss Inc. N.Y. Ed. Etzler, M. 138, 7-20 (1983).

21. Hoebeke, J. and Strosberg, A.D. "Physico-chemical studies of β-adrenergic receptors on intact cells". In "Investigation of membrane-located receptors". Guildford. Ed. E. Reid, 389-395 (1984).

22. Strosberg, A.D., "Anti-idiotypic antibodies as immunological internal images of hormones" In "Idiotypy". Eds. Köhler, H., Urbain, J. and Cazenave, P.A. Academic Press, 365-383 (1984).

23. Hoebeke, J., Durieu, O., Delavier, C., Schmutz, A. et Strosberg, A.D. "Biochemical and immunological studies of β-adrenergic receptors on various cell types". 5th International Conference on Cyclic Nucleotides and Protein Phosphorylation. Eds. Raven Press 17, 77-80 (1984).

24. Strosberg, A.D. "Purification of plasma membrane proteins by affinity chromatography". In "Membrane receptor purification and characterization techniques" Ed. Venter, Alan R. Liss N.Y., 1-13 (1984).

25. Strosberg A.D. and Schreiber A.B. "Antibodies to Receptors and idiotypes as Probes for Hormone and Neurotransmitter Receptor Structure and Function" in "Antibodies to receptors" Ed. M.F. Greaves, 15-42 (1984).

26. Strosberg, A.D., Chamat, S., Guillet, J.G., Schmutz, A., Durieu, O., Delavier, C. and Hoebeke, J. "New immunological tools for the study of β-adrenergic receptors" in "Monoclonal anti-idiotypic antibodies probes for receptor structure and function". Alan Liss, Ed. Venter, C. and Lindstrom, E.S., 151-162 (1984).

27. Legionella monoclonal antibody", in "Legionella", Ed. ASM Washington USA, Second Symposium Legionella Atlanta, pp. 39-49 (1984).

28. Strosberg, A.D., Delavier-Klutchko, C., Cervantes, P., Guillet, J.-G., Schmutz, A., Kaveri, S., Durieu-Trautmann, O. and Hoebeke, J. "Biochemical and immunological studies of β_1- and β_2-adrenergic receptors". In "Adrenergic Receptors : Molecular Properties and Therapeutic Implications", Eds. R.J. Lefkowitz and E. Lindenlaub, Symposium Saint-Paul de Vence, F.K.S. Schattauer Verlag, Stuttgart-New York, 53-80 (1984).

29. Petitjean, F., Guillet, J.-G., Vray, B., Hoebeke, J., Tram, C. and Strosberg, A.D. "Standardization for diagnostic purposes of a monoclonal antibody against Legionella pneumophila", Devel. Biol. Standard., 99-105 (1985).

30. Strosberg, A.D., Guillet, J.G., Chamat, S. and Hoebeke, J. "Recognition of physiological receptors by anti-idiotypic antibodies : molecular mimicry of the ligand or cross reactivity?". In Current Topics in Microbiology and Immunology "Images of Biologically active structures in the Immune System", Springer-Verlag, H. Koprowski and F. Melchers Eds., 92-110 (1985).

31. Hoebeke, J., Guillet, J.G., Chamat, S. and Strosberg, A.D. "Anti-idiotype antibodies for the study of membrane receptors: the double monoclonal antibody approach" in "Investigation and Exploitation of Antibody Combining Site". Ed. E. Reid, Plenum Press, London, 115-122 (1985).

32. Petitjean, F., Guillet, J.-G., Vray, B., Hoebeke, J. and Strosberg, A.D. "ELISA test using monoclonal antibody for the detection of Legionella pneumophila antigen" in "Methods in Enzymatic Analysis" XI. Eds. H.U. Bergmeyer, M. Grassl, Verlag Chimie, Weinheim (FRG), 173-188 (1986).

33. Strosberg, A.D., Buffard, D., Lauwereys, M. and Foriers, A. "Legume lectins: a large family of homologous proteins", Liener, I., Sharon, N. and Goldstein, I.J. Eds, in "The Chemistry and Biology of Lectins", 250-264 (1986).

34. Strosberg, A.D. "The role of antibodies in autoimmune and other disorders : an introduction". In "Clinical Use of Intravenous Immunoglobulins". A. Morell and U.E. Nydegger Eds, Academic Press, 413-420 (1986).

35. Marullo, S. and Strosberg, A.D. "Role of receptor-binding antibodies and anti-idiotypic antihormone antibodies in immune regulation". In Concepts Immunopathol., J. M. Cruse Ed., Vol. 3, 176-192 (1986).

36. Strosberg, A.D. "Affinity chromatography of proteins". In "Practical Protein Chemistry - A Handbook", A. Darbre Ed., John Wiley and Sons Ltd, 166-180 (1986).

37. Strosberg, A.D., Buffard, D., Kaminski, P.A., Chapot, M.P., Rossow, P.W. and Foriers, A. "Lectin multigene families in leguminous and non-leguminous plants". In "Molecular Biology of Seed Storage Proteins and Lectins". L.M. Shannon and M.J. Crispeels Eds, American Society of Plant Physiologists, 1-16 (1986).

38. Strosberg, A.D., Delavier-Klutchko, C., Cervantes, P., Guillet, J.G., Schmutz, A., Kaveri, S., Durieu-Trautmann, O. and Hoebeke, J. "Biochemical and immunological studies of $ß_1$- and $ß_2$-adrenergic receptors". In "Adrenergic Receptors : Molecular Properties and Therapeutic Implications". R.J. Lefkowitz and E. Lindenlaub Eds, F.K. Schattauer Verlag, Stuttgart, 53-81 (1986).

39. Strosberg, A.D., Chamat, S., Guillet, J.G., Nahmias, C., Hoebeke, J. and Emorine, L. "Idiotypy of ß-adrenergic ligand binding antibodies and receptors". In "Idiotypes", M. Reichlin and J.D. Capra Eds, Academic Press Inc., 139-155 (1986).

40. Strosberg, A.D. "Receptors and recognition : from ligand binding to gene structure". In "Oncogenes and Growth Factors", R.A. Bradshaw and S. Prentis Eds., Elsevier Publishers, 201-209 (1987).

41. Strosberg, A.D. "Immunological tools for the study of plasma membrane receptors". In "Biomembrane and receptors mechanisms", Vol. 7, Fidia Research Series, E. Bertoli, D. Chapman, A. Cambria and V. Scapagnini Eds. Liviana Press, Padova, Springer Verlag, 319-324 (1987).

42. Strosberg, A.D. "Antiidiotypes and Receptors". In "Excerpta Medica International Congress Series", Proceedings of the 1st IUIS Conference on Clinical Immunology, Toronto, W. Pruzanski, M. Seligmann Eds, Elsevier Science Publishers BV, 21-34 (1987).

43. Strosberg, A.D. "The ß-Adrenergic Receptors and G-Proteins". In "The Molecular Biology of Receptors" A.D. Strosberg Ed., Ellis Horwood Ltd, Chichester, England. 139-163 (1987).

44. Emorine, L.J., Nahmias, C., Marullo, S., Delavier-Klutchko, C. Kaveri, S.V., Durieu- Trautmann, O. and Strosberg, A.D. "Structure of the gene for the human $ß_2$-adrenergic receptor". In the Proceedings of the VIth International Catecholamine Symposium, Jerusalem. Neurology and Neurobiology Vol. 42, A. Dahlström, R.H. Belmaker and M. Sandler Eds, Alan R. Liss Inc. New York, 345-349 (1988).

45. Thuan, B.P., Strosberg, A.D. and Hoebeke, J. "Purification and structural properties of the lectins from *Tachyleus tridentatus*". In the Proceedings of Interlec 9 on Lectins, Cambridge, 405-410 (1988).

46. Strosberg, A.D. "New developments in monoclonal antibody technology". In "Monoclonal antibodies against human red blood cell markers" Ph. Rouger and Ch. Salmon Eds., Arnette, France, 7-21 (1988).

47. Couraud, P.-O. and Strosberg, A.D. "Anti-receptor anti-idiotypic antibodies". In Molecular Neuroanatomy", Van Leeuwen, Buijs, Pool and Pach (Eds.), Elsevier Science Publishers B.V., 191-203 (1988).

48. Emorine, L., Marullo, S., Briend-Sutren, M.M., Delavier-Klutchko, C., Eshdat, Y., Guillet, J.G., Patey, G. and Strosberg A.D. "Molecular physiology of adrenergic receptors" Proc. Symposium "Progress in Asthma and COPD" Elsevier, Science Publishers BV, Crête, 113-121 (1989).

49. Emorine, L., Marullo, S., Sutren, M.M., Delavier, C.,Eshdat, Y., Raposo, G., and Strosberg, A.D "Common properties receptors to GTP binding regulatory proteins" In "Molecular Biology of Neuroreceptors and Ion Channels" Santorini - Nato summer conference, NATO ASI Series, Vol. H32, 246-257 (1989).

50. Van Huizen, F., Shaw, C., Strosberg, A.D., and Cynader, M.S. "localisation of muscarinic acetylcholine receptors in cas visual cortex during postnatal development" In "Molecular Biology of Neuroreceptors and Ion Channels" Santorini - Nato summer conference, NATO ASI Series, Vol. H32 (1989).

51. Strosberg, A.D. "Biologie Moléculaire des Récepteurs". In Pharmacologie Moléculaire", Y. Landry et J.P. Gries Eds., Medsi, McGraw Hill Paris, 81-97 (1989).

52. Fougereau, M., Mazza, G., Nahmias, C., Gonzales, R., Avrameas, S., Strosberg, A.D. and Schiff, C. "Idiotypic cross-reactivity and regulation of the immune system". In "Idiotype networks in Biology and Medicine", Urbain and UytdeHaag Eds, Elsevier, Amsterdam (1989).

53. Durieu-Trautmann, O.and Delavier-Klutchko, C. "Etude d'un récepteur membranaire des catécholamines couplé à l'adénylate cyclase" in "Techniques des Interactions Ligands-récepteurs", M. Daëron et A.D. Strosberg Eds, ociété Française d'Immunologie , Editions INSERM, 41-51 (1989).

54. Guillet, J.G., Lengagne, R. and Briner T. "nature du ligand reconnu par les lymphocytes T auxiliaires" in Techniques d'Etude des interactions Ligands-récepteurs", M. Daëron et A.D. Strsoberg Eds. Société Française d'Immunologie, Editions INSERM, 41-51 (1989).

55. Zilles, K., Schröder, H., Schröder, U., Horvath, E., Werner, L., Luiten, P.G.M., Maelicke, A. and Strosberg, A.D. "Distribution of cholinergic receptors in the rat and human neocortex" in "Central cholinergic synpatic transmission", M. Frotcher and U. Misgled Eds, Birkhäuser Verlag Basel, Boston, Berlin, 212-228 (1989).

56. Emorine, L.J. and Strosberg, A.D. "Molecular analysis of the three human ß-adrenergic receptors" Adrenoceptors : Structure, Mechanisms, Function in Advances in Pharmacological Sciences, Eds E. Szabadi, Birkhäuser Verlag Basel Publishers, 79-89 (1991).

57. Schröder, H., Giacobini, E., Struble, R.G., Zilles, K., Maelicke, A., Luiten, P.G.M. and Strsoberg, A.D."cellular Distribution and Expression of Cortical Acetylcholine Receptors in Aging and Alzheimer 's Disease" in Aging and Alzheimer's Disease. Sensory systems, Neuronal Growth and Neuronal

Metabolism. Annals of the N.Y. Academy of Sciences.J.H. Growdon, S. Corkin, E. Ritter-Walker & R.J. Wurtman Eds. Vol. 640, 189-192.(1991).

58. Hoebeke, J. and Strosberg, A.D. " Drug as Antigens" in "Structure of Antigens" Ed. M. Van Regenmortel Telford Press, Vol. 1 Chapter 12, 261-291 (1992).

59. Strosberg, A.D. "Biotechnology of the ß-adrenergic receptors" Molecular Neurobiology, 4, 211-250 (1992) .

60. Strosberg, A.D. "Anti-receptor antibodies as ligands" in " Receptor-ligand interactions, a practical approach", Chapter 2, 20-36 (1992)

61. Ravet, V., Blin, N., Guillaume, J-L., Petitjean, F., Cabanié, L. and Strosberg, A.D. "High level functional expression of human ß1-adrenergic receptor in baculovirus-infected cells screened by a rapid *in situ* procedure" Journal of Receptor Research Vol.13, issue 1 541-558 (1993)

62. Strosberg, A.D., Camoin, L., Blin, N. and Maigret, B. "In receptors coupled to GTP-binding proteins, ligand binding and G-protein activation is a multistep dynamic process" Drug Design & Discovery, Symposium on the Neuromedical Chemistry : G-proteins coupled receptors, Lund May (1992).

63 Durieu-Trautmann, Bourdoulous, S., Roux, F., Bourre, J-M., Strosberg, A.D. and Couraud, P-O. "Immortalized rat brain microvessel endothelial cells : II-Pharmacological characterization" frontiers in Cerebral Vascular Biology "Transport and its regulation" Ed. L.R. Drewes and A.L. Betz, N.Y. 1993 pp. 205-210 (1992).

64 Strosberg, A.D. "Structure, function and regulation of the ß3-adrenergic receptors" Pennington Nutrition Series, 5, (1994).

65 Strosberg, A.D. "Structure, function and regulation of the ß-adrenergic receptor subtypes" Critical Reviews in Pharmacology, chapter 15, 123-127 (1994).

66 Strosberg, A.D. "Recombinant receptors as probes for the study of genetic stability" in "Genetic Stability and Recombinant Product Consistency" Brown F, Lubiniecki AS (Eds) in Dev. Biol. Stand. Basel, Karger Vol. 83, 153-157 (1994).

67 Pietri-Rouxel, F., Drumare, M.F. and Strosberg, A.D. "Pharmacological characteristics of ß3-adrenergic receptor" Pharmacology Communications, Special Issue "Pharmacology of Adrenoceptors" Vol 6, 229-236, Harwood Academic Publishers, Eds N.G. Bowery and R.R. Ruffolo (1995).

68 Strosberg, A.D. "Structure and function of the β_3-adrenoceptor. Proceedings of the 7th ICO Meeting, Toronto (1995).

69 Hoebeke, J., Guillet, J.G. and Strosberg, A.D."Use of receptors expressed in Escherichia coli to study autoimmunity against G protein-coupled membrane proteins" in Studies of functional domains of receptor and channels, Chapter 18 in Methods in Neurosciences, Academic Press Inc. Vol. 25 pp. 345365 (1995).

70 Strosberg, A.D. "Structural and functional diversity of β-adrenergic receptors" in Diversity of interacting receptors, Annals of the New York Academy of Sciences Vol. 27 (April 1995) 253-260 (1995).

71 Strosberg, A.D. "Structure and function of the β_3-adrenoceptor" Progress in Obesity Research (Eds A.A.H. Anderson, C. Bouchard, D. Lau, L. Leiter and R. Mendelson) John Libbey & Cie Ltd Chap.18, 133-140 (1996).

72 Nahmias, C., Cazaubon, S., Sutren, M.M., Masson, M., Lazard, D., Villageois, P., Elbaz, N. & Strosberg, A.D. "Molecular and functional characterization of angiotensin II AT2 receptor in neuroblastoma N1E-115 cells" Recent Advances in Cell. and Mol. Aspects of Angiotensin Rec. Chap. 17, 167-173 (1996).

73 Strosberg, A.D. "G-protein coupled"R7G" receptors" Cell Signalling in Cancer Survey (P.J. Parker & T. Pawson, Guest Eds, J. Tooze Imperial Cancer Research Fund Publishers: 27, 65-83 (1997).

74 Strosberg, A.D. & Camoin, L. "Studies of β3-agonists in model systems and in immortalized human and animal adipose tissue" in Obesity II Recent Advances in Understanding and Treatment IBC Library Series (C.A. Thibeault and L.M. Savage Eds) Chap. 2.2., 47-68 (1997).

75 Strosberg, A.D. "Advances in the Understanding of the Adipose Cell" in Obesity II Recent Advances in Understanding and Treatment IBC Library Series (C.A. Thibeault and L.M. Savage Eds) Chap. 5.1.,229-231 (1997).

76 Strosberg, A.D. "Structure and function of the β3-adrenoceptor" in "Advances in Pharmacology" Catecholamines Bridging Basic Science with Clinical Medicine, (D.S. Goldstein, G. Eisenhofer and R. McCarty Eds, Academic Press, Vol. 42, 511-513 (1997).

77 Strosberg, A.D. & Fernandes P.B. "Recombinant G protein-coupled receptors as screening tools for drug discovery" in "Receptor-Based Drug Design" Chapter 14, pp345-361 (M. Dekker, Eds) (1998).

78 Strosberg, A.D. "The β3-adrenoreceptor" Edited by A.D.Strosberg, (Taylor & Francis, London UK) (2000)

79. Strosberg, A.D. "Genomics/Functional Proteomics for Identification of New Targets" in Handbook of Drug Screening, Chapter 17, 459-471 (Marcel Dekker, Inc. Eds, New York, USA) (2001).

80 Strosberg A.D., Daviet L., Borg-Capra C. "Functional Proteomics Applied to Obesity Research" in "Progress in Obesity Research", pp 229-233, G. Medeiros-Neto, A. Halpern and C. Bouchard Eds, John Libbey Eurotext Ltd.(2003).

RESEARCH

Normalization and Subtraction: Two Approaches to Facilitate Gene Discovery

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Large-scale sequencing of cDNAs randomly picked from libraries has proven to be a very powerful approach to discover (putatively) expressed sequences that, in turn, once mapped, may greatly expedite the process involved in the identification and cloning of human disease genes. However, the integrity of the data and the pace at which novel sequences can be identified depends to a great extent on the cDNA libraries that are used. Because altogether, in a typical cell, the mRNAs of the prevalent and intermediate frequency classes comprise as much as 50–65% of the total mRNA mass, but represent no more than 1000–2000 different mRNAs, redundant identification of mRNAs of these two frequency classes is destined to become overwhelming relatively early in any such random gene discovery programs, thus seriously compromising their cost-effectiveness. With the goal of facilitating such efforts, previously we developed a method to construct directionally cloned normalized cDNA libraries and applied it to generate infant brain (INIB) and fetal liver/spleen (INFLS) libraries, from which a total of 45,192 and 86,088 expressed sequence tags, respectively, have been derived. While improving the representation of the longest cDNAs in our libraries, we developed three additional methods to normalize cDNA libraries and generated over 35 libraries, most of which have been contributed to our Integrated Molecular Analysis of Genomes and Their Expression (IMAGE) Consortium and thus distributed widely and used for sequencing and mapping. In an attempt to facilitate the process of gene discovery further, we have also developed a subtractive hybridization approach designed specifically to eliminate (or reduce significantly the representation of) large pools of arrayed and (mostly) sequenced clones from normalized libraries yet to be (or just partly) surveyed. Here we present a detailed description and a comparative analysis of four methods that we developed and used to generate normalize cDNA libraries from human (15), mouse (3), rat (2), as well as the parasite *Schistosoma mansoni* (1). In addition, we describe the construction and preliminary characterization of a subtracted liver/spleen library (INFLS-S1) that resulted from the elimination (or reduction of representation) of ~5000 INFLS–IMAGE clones from the INFLS library.

Large-scale single-pass sequencing of cDNA clones randomly picked from libraries has proven to be a powerful approach to discover genes (Adams et al. 1991, 1993a,b, 1995; Khan et al. 1992; McCombie et al. 1992; Okubo et al. 1992; Matsubara and Okubo 1993; see also Hillier et al., this issue). However, the significance of using cDNA libraries that are well suited for this purpose should not be underestimated (Adams et al. 1993b).

Ordinary cDNA libraries may contain a high frequency of undesirable ("junky") clones (Adams et al. 1991, 1992) that may not only drastically impair the overall efficiency of the approach, but also seriously compromise the integrity of the data that are generated. Among such junky clones are: (1) clones that consist exclusively of poly(A) tails of mRNAs; (2) clones that contain very short cDNA inserts; (3) clones that contain nothing but the 3′ half of the *Not*I–oligo(dT)$_{18}$ primer used for synthesis of first-strand cDNA ligated to an adaptor; and (4) chimeric clones, i.e., cDNAs derived from different mRNAs joined artifactually during ligation . Furthermore, given that, as a general rule, the frequency of occurrence of a cDNA clone in a library is equivalent to that of its corresponding mRNA in the cell, even high-quality cDNA libraries may not be ideal for large-scale sequencing.

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Reassociation–kinetics analysis indicates that the mRNAs of a typical somatic cell are distributed in three frequency classes: (1) superprevalent (consisting of about 10–15 mRNAs that altogether represent 10–20% of the total mRNA mass); (2) intermediate (1000–2000 mRNAs; 40–45%); and (3) complex (15,000–20,000 mRNAs; 40–45%) (Bishop et al. 1974; Davidson and Britten 1979). Accordingly, once most mRNAs of the prevalent and intermediate frequency classes are identified, redundancy levels are expected to become greater than 60%. For this reason, the use of normalized libraries, in which the frequency of all clones is within a narrow range (Soares et al. 1994), has been shown to be beneficial for large-scale sequencing (Berry et al. 1995; Houlgatte et al. 1995). Calculations show that at $C_0t = 5.5$ [where C_0 is the total DNA concentration and t is the time (moles nucleotides per liter × sec)], of the three kinetic classes of mRNAs, the most abundant species are diminished drastically, while all frequencies are brought within the range of one order of magnitude (Soares et al. 1994).

However, because a large fraction of all human genes has been identified already, redundant identification of genes that are expressed in multiple tissues cannot be avoided simply by the use of normalized libraries. Hence, we argue that the use of subtractive cDNA libraries enriched for genes expressed at low levels and that have not yet been identified should become increasingly more advantageous for large-scale sequencing programs.

While attempting to improve the representation of the longest cDNAs in our libraries, we developed three methods for construction of normalized libraries, in addition to the procedure that we described previously (Soares et al. 1994), and used them successfully to generate normalized cDNA libraries from human (15), mouse (3), rat (2), and *Schistosoma mansoni* (1) tissues. All human and mouse cDNA libraries have been contributed to the Integrated Molecular Analysis of Genomes and Their Expression (IMAGE) Consortium (Lennon et al. 1996), and to date a total of 315,408 expressed sequence tags (ESTs) have been derived from these libraries (dbEST release 052396; http://www.ncbi.nlm.nih.gov).

Here we present a detailed description and a comparative analysis of the four methods that we have developed to normalize cDNA libraries; we describe a simple procedure for the construction of subtractive cDNA libraries; and we discuss strategies that take advantage of subtractive hybridization to expedite the ongoing IMAGE/Washington University/Merck gene discovery program.

RESULTS

While attempting to improve the representation of the longest cDNAs in our normalized libraries, we developed four methods and constructed over 35 libraries, most of which are described here. A list comprising 15 human, three mouse, two rat, and one schistosome library with their respective names, number of recombinants, sequence tags, and methods used for normalization and preparation of single-stranded plasmids is shown in Table 1.

Extensive characterization of two normalized libraries [normalized infant brain (1NIB) and normalized fetal spleen (1NFLS)] constructed according to our previously described procedure (Soares et al. 1994; here designated as method 1) confirmed our original observations that a great extent of normalization can be achieved with this method for most cDNA species (e.g., cf. lanes 9,10 in Fig. 1M–P). It is noteworthy that the frequency of cDNA 122 (used as the probe in P) was increased with normalization from <0.0006% in the starting library to 0.007% in the 1NIB library (Soares et al. 1994). However, Southern hybridization of starting and normalized libraries with a battery of cDNA probes revealed that on occasion truncated clones were favored over their longest counterparts during the process. This was first observed when Southern blots of *NotI* + *HindIII*-digested plasmid DNA from starting and normalized infant brain libraries were hybridized with a cDNA probe for mitochondrial 16S rRNA (see Fig 1L, lanes 9,10). Not only was the frequency of these mitochondrial cDNA clones not reduced effectively during the process of normalization (frequency of occurrence in starting and normalized infant brain libraries was 1.4% and 1.0%, respectively), but also the length of the hybridizing cDNAs was noticeably smaller in the normalized library: Comparative sequence analysis (not shown) of a number of hybridizing mitochondrial 16S rRNA clones from both starting and normalized libraries revealed that whereas the 3′ end of most cDNAs derived from the starting library corresponded to the bona fide 3′ end of the 16S rRNA, the 3′ end of the majority of the cDNAs isolated from the normalized library corresponded to sequences further upstream on the

Table 1. Complete List and Main Features of the Normalized Human, Mouse, Rat, and Schistosome cDNA Libraries

mRNA source	Normalized library name	Number of recombinants in the normalized library	Preparation of single-stranded plasmids	Method of normalization	Library tag[a]
Human infant brain[b]	1NIB	2,500,000	in vivo	1	AGGAA
Human fetal liver spleen[c]	Nb2HFLS20W (1NFLS)	19,000,000	in vivo	1	AGATCT
	5Nb2HFLS20W	3,200,000	in vitro	2-1	
	6Nb2HFLS20W	1,400,000	in vitro	2-3	
	14Nb2HFLS20W	3,200,000	in vitro	4	
	15Nb2HFLS20W	35,000,000	in vitro	2-2	
Human term placenta	Nb2HP	750,000	in vivo	2-1	AGGAA
Human 8-9W placenta	2NbHP8-9W	100,000	in vitro	2-3	GA
Human breast[d]	2NbHbst-3NbHBst[e]	2,090,000	in vivo	2-1	CC
Human adult brain[f]	N2b4HB55Y-N2b5HB55Y[g]	3,170,000	in vivo	2-1	GC
Human retina[h]	2N2b4HR-N2b5HR	1,600,000	in vivo	2-1	AC
Human pineal gland[i]	3NbHPG	1,000,000	in vitro	2-1	CG
Human ovary tumor[j]	NbHOT	1,100,000	in vivo	2-1	GG
Human melanocytes[k]	2NbHM	6,800,000	in vitro	2-3	AG
Human fetal heart[l]	NbHH19W	9,700,000	in vitro	4	ATC
Human parathyroid adenoma[m]	NbHPA	3,400,000	in vitro	4	ACCAA
Human senescent fibroblast[n]	NbHSF	9,900,000	in vitro	4	AACCA
Human multiple sclerosis plaques[o]	2NbHMSP	1,100,000	in vitro	3	CA
Human fetal lung[l]	NbHL19W	21,700,000	in vitro	4	AA
19.5-dpc mouse embryo[p]	p3NMF19.5	3,400,000	in vitro	4	ACAAC
17.5-dpc mouse embryo[p]	NbME17.5	6,800,000	in vitro	4	GACAC
13.5- to 14.5-dpc mouse embryos[p]	NbME13.5-14.5	380,000	in vitro	4	GGAAA
Rat heart[q]	NbRH	400,000	in vitro	4	ACAAC
Rat kidney[q]	2NbRK	130,000	in vitro	4	CAAAC
8-week-old adult schistosome[r]	NbS8W	1,000,000	in vitro	4	GAAAG

With the exception of 1NIB, which was constructed in the Lafmid BA vector, all libraries were constructed in the pT7T3–*Pac* vector. Cloning sites were *Not*I and *Eco*RI, except for fetal liver spleen (*Pac*I and *Eco*RI) and infant brain (*Not*I and *Hind*III).

[a]The library tag is a sequence identifier present in the oligonucleotide used to prime the synthesis of first-strand cDNA, between the recognition sequence for the rare restriction enzyme (*Not*I or *Pac*I in the case of the liver spleen library) used for directional cloning and the dT_{18} stretch (or dT_{25} in the human parathyroid adenoma, senescent fibroblast, mouse embryo, rat, and *Schistosoma mansoni* libraries) located at the 3′ end of the primer.

[b]Human infant brain (kindly provided by Dr. Conrad Gilliam, Columbia University, New York, NY) was from a 72-day-old female who died in consequence of spinal muscular atrophy.

[c]Human fetal liver spleen (kindly provided by Dr. Stephen Brown, Columbia Presbyterian Medical Center, New York, NY) was from a 20-week-old postconception normal female.

[d]Total cellular poly(A)$^+$ mRNA from normal breast pooled from reduction mammoplasty tissue was kindly provided by Dr. Anne Bowcock and Ms. Monique Spillman, University of Texas Southwestern Medical Center at Dallas.

[e]2NbHbst differs from 3HbHbst in the C_0t used for hybridization (23′ and 20, respectively).

[f]Total cellular adult brain RNA (kindly provided by Dr. Donald Gilden, University of Colorado Health Sciences Center, Denver) was obtained from a 55-year-old male who died of a ruptured aortic aneurysm. Brain tissue (frontal, parietal, temporal, and occipital cortex from the left and right hemispheres, subcortical white matter, basal gangila, thalamus, cerebellum, midbrain, pons, and medulla) was acquired 17-18 hr after death.

[g]N2b4HB55Y and 2N2b4HR differ from N2b5HB55Y and N2b5HR, respectively, in the average size of their cDNA inserts (1.5–2.5 kb and 0.4–1.5 kb, respectively).

[h]Total cellular normal human retina RNA (kindly provided by Dr. Roderick R. McInnes, University of Toronto and Hospital for Sick Children, Canada) was obtained from a 55-year-old Caucasian male.

[i]Human pineal gland [kindly provided by Dr. David Klein, National Institute of Child Health and Human Development, National Institutes of Health (NIH)] was derived from a group of three pineal glands (gland 1: 48-year-old Caucasian male: gland 2: 18-year-old Caucasian female, gland 3: 20-year-old African American male).

[j]Total cellular human ovary tumor mRNA was kindly provided by Dr. Anne Bowcock and Ms. Monique Spillman, University of Texas Southwestern Medical School. It was obtained from a 36-year-old Caucasian with a papillary serous cystadenocarcinoma grade III with surface extensions and metastases.

[k]Total cellular human melanocyte RNA (kindly provided by Dr. Anthony Albino and Dr. Alice de Oliveira, Memorial Sloan-Kettering Cancer Center, New York, NY) was derived from normal foreskin.

[l]Normal human fetal heart and lung (kindly provided by Dr. Stephen Brown, Columbia Presbyterian Medical Center) were derived from the same 19-week-postconception specimen.

[m]Human parathyroid tumor (kindly provided by Dr. Stephen Marx, National Institute of Diabetes and Digestive and Kidney Diseases, NIH) was derived from sporadic adenomas.

[n]Cytoplasmic mRNA from senescent normal human fibroblasts was kindly provided by Dr. Barbara Burhart (National Institute of Environmental Health Sciences, NIH). The cells were prepared by passaging normal human fibroblasts derived from foreskin until they exhibited an enlarged, flattened phenotype and failure to divide (labeling index of <2% following 48 hr-bromodeoxyuridine incorporation).

[o]Total cellular RNA from multiple sclerosis plaques (kindly provided by Dr. Kevin G. Becker, NINDS, NIH) was extracted from four lesions obtained from one patient.

[p]Total cellular RNA for construction of the mouse (C57BL/6J strain) embryonic libraries was kindly provided by Dr. Minoni Ko (Wayne State University, Detroit, MI).

[q]Rat tissues were obtained from an adult ZMc-Miller Sprague Dawley female and were kindly provided by Dr. Stephen Brown (Columbia Presbyterian Medical Center).

[r]Total cellular RNA from mature 8-week-old *Schistosoma mansoni* worms was kindly provided by Dr. Ron Blanton, Case Western Reserve University, Cleveland, OH.

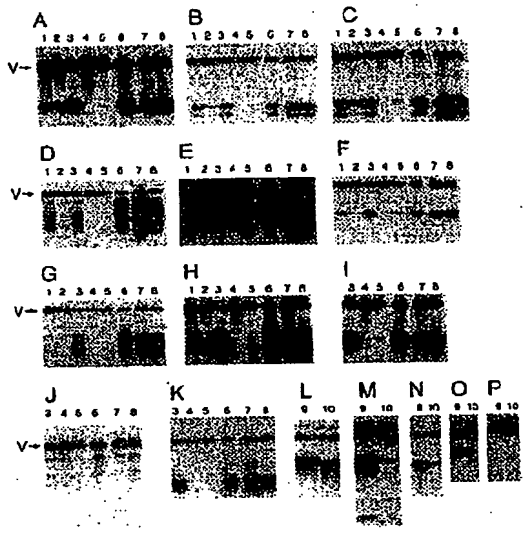

Figure 1 Comparative analysis of starting and normalized cDNA libraries by Southern hybridization with 14 cDNA probes. The 0.015 μg *Pad* + *EcoRI* digested plasmid DNA from the starting fetal liver/spleen library (lane *6*), from the normalized fetal liver/spleen libraries constructed according to method 2-1 (lane *1*), method 2-3 (lane *2*), method 2-2 (lane 3), method 1 (lane *4*), method 4 (lane *5*), and from the liver/spleen mini-libraries enriched for abundant cDNAs (HAP-bound fractions) generated with method 2-1 (lane *7*) and method 4 (lane *8*) were electrophoresed on 1% agarose gels, transferred to nylon membranes (GeneScreenPlus; DuPont/NEN) and hybridized at 42°C in 50% formamide, 5 × Denhardt's solution, 0.75 M NaCl, 0.15 M Tris (pH 7.5), 0.1 M sodium phosphate, 0.1% sodium pyrophosphate, 2% SDS containing sheared and denatured salmon sperm DNA at 100 μg/ml. Similarly, 0.05 μg *NotI* + *HindIII* digested plasmid DNA from the starting (IB; lane *9*) and normalized (1NIB; lane *10*; method 1) infant brain libraries (Soares et al. 1994) were electrophoresed, transferred, and hybridized as described above. Radioactive probes were prepared by random primed synthesis using the Prime-It II kit (Stratagene). The following probes were used: α-globin (*A*), β-globin (*B*), γ-globin (*C*), serum albumin (*D*, shorter exposure; *E*, longer exposure), acidic ribosomal phosphoprotein PO (*F*), H19 RNA (*G*, shorter exposure; *H*, longer exposure), apolipoprotein A (*I*), angiotensinogen (*J*), unknown cDNA 8 (*K*), mitochondrial 16S rRNA (*L*), α-Tubulin (*M*), myelin basic protein (*N*), secretogranin (*O*), and unknown cDNA 122 (*P*). All probes were contaminated intentionally with a small amount of vector DNA to enable visualization of vector bands and thus confirm that a similar amount of library DNA was loaded in all lanes. (V) vector band, which is released from the cDNA inserts by double digestion with the restriction enzymes specified above.

16S rRNA. The occurrence of such 3' truncations was also documented by sequence analysis (not shown) for serum albumin cDNAs in the fetal liver/spleen library (see Fig. 1D,E, lanes 4,6).

Reasoning that this problem could be circumvented if the fragments used in the hybridization with the single-stranded circles (1) were in excess, and (2) spanned the entire length of the cDNAs, we developed an alternative procedure to normalize cDNA libraries based on hybridization of in vitro synthesized RNA (driver) from an entire library with the library itself in the form of single-stranded circles (tracer) (see methods 2-1 and 2-2 in Fig. 2). Several normalized libraries were generated by this procedure (see Table 1).

Southern hybridization of endonuclease-restricted plasmid DNA from starting and normalized libraries with a number of cDNA probes (Fig. 1) indicated clearly that these methods effectively improved the representation of the longest cDNAs in the normalized libraries (e.g., cf. lanes 1,4 in Fig. 1A,D,E,G,H). However, characterization of one of these libraries (SNb2HFLS20W) by colony hybridization with cDNA probes (not shown) indicated that this approach was effective to reduce the frequency of some, but not all, of the most abundant clones (e.g., serum albumin was reduced about 20-fold, whereas γ-globin was reduced only twofold). No difference was observed when hybridizations were performed at different conditions [0.4 M NaCl and 50% formamide at 42°C as in methods 2-1 and 2-3; 0.12 M NaCl, 50% formamide, and 1% sodium dodecyl sulfate (SDS) at 30°C as in method 2-2 (see lane 3 in Fig. 1); 0.4 M NaCl and 80% formamide at 42°C, not shown].

It is noteworthy that Northern hybridization (not shown) of in vitro transcribed RNA synthesized from an entire plasmid library with probes derived from the abundant cDNAs that failed to be normalized effectively by this procedure (e.g., globins in the fetal liver/spleen library and glyceraldehyde-3-phosphate dehydrogenase (G3PD) in the breast library) indicated that they were not as prevalent in the population of in vitro transcribed RNAs as they were in their respective starting cDNA libraries.

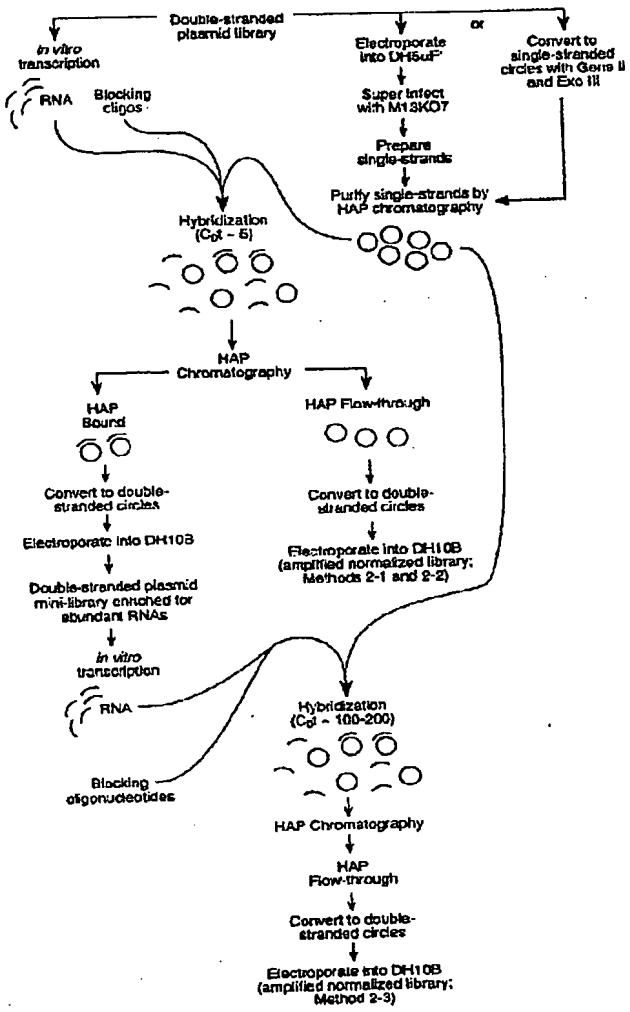

Figure 2 Diagram of the normalization methods 2-1, 2-2, and 2-3. Double-stranded plasmid DNA representing an entire starting library is (1) linearized with either *Sfi*I, *Not*I, or *Pac*I and used as template for synthesis of RNA in vitro using T3 or T7 RNA polymerases, and (2) converted to single-stranded circles either in vivo, upon electroporation into DH5αF' and superinfection with M13KO7, or in vitro by the combined action of Gene II and Exonuclease III (Life Technologies). Single-stranded plasmid DNA is HAP-purified and hybridized (C_0t ~ 5) with excess RNA (pretreated with RNase-free DNAse I; Promega), blocked with appropriate oligonucleotides to prevent hybridization through common vector sequences (see Methods section). Both the fraction that remains single-stranded (flow-through) as well as the resulting hybrids (bound) are purified by HAP chromatography. The HAP flow-through fraction is converted to double-stranded plasmids, electroporated into DH10B bacteria (Life Technologies), and propagated under ampicillin selection to generate an amplified normalized library (methods 2-1 and 2-2, depending on the conditions used for hybridization; see Methods section). The HAP-bound fraction is also converted similarly to double-stranded plasmids, electroporated into bacteria, and propagated under ampicillin selection to generate a mini-library enriched for abundant cDNAs. Double-stranded plasmid DNA from this mini-library is linearized and used as template for synthesis of RNA in vitro. After digestion of the plasmid DNA template with ribonuclease-free DNAse I (Promega), the RNA (driver) is blocked with appropriate oligonucleotides and hybridized (C_0t ~100–200) with HAP-purified single-stranded plasmids derived from the starting library (see above). The remaining single-stranded circles are purified by HAP chromatography, converted to double-stranded circles, electroporated into DH10B (Life Technologies), and propagated under ampicillin selection to generate an amplified normalized library (method 2-3).

A significantly improved extent of normalization was achieved when runoff RNA synthesized from the plasmid mini-library enriched for abundant cDNAs (hydroxyapatite (HAP)-bound fraction of method 2-1 in Fig. 2) was hybridized (C_0t = 100–200) with single-stranded circles from the starting library (see method 2-3 in Fig. 2 and Table 1; cf. lanes 1,2 in Fig. 1A–D,F,G).

In an effort to preserve the positive characteristics of both methods 1 and 2 (i.e., the adequate extent of normalization achieved with method 1, and the improved representation of the longest cDNAs achieved with method 2), we developed two additional reassociation kinetics based procedures involving DNA–DNA hybridization (methods 3 and 4; see Fig. 3).

Method 3, which was successfully used to construct a normalized library from multiple

sclerosis plaques (see 2NbHMSP in Table 1), involved hybridization of a 20-fold excess of single-stranded cDNA fragments (comprising the 5′ halves of all inserts of the starting library, generated by Exonuclease III digestion of gel-purified double-stranded cDNAs; see Fig. 3) with complementary single-stranded circles produced in vitro by the combined action of Gene II and Exonuclease III (Life Technologies).

Southern hybridization of *NotI* + *Eco*RI-digested plasmid DNA from the starting and normalized (with methods 2-1 and 3) multiple sclerosis plaques library with mitochondrial 16S rRNA and myelin basic protein cDNA probes (not shown) clearly indicated that method 3 was superior to method 2-1 in that a much greater extent of normalization was achieved, at the same time that it maintained (similar to method 2-1) appropriate representation of the longest cDNAs in both cases.

For the libraries constructed with method 4 (see Table 1 and Fig. 3), double-stranded cDNA inserts generated by the polymerase chain reaction (PCR) with T3 and T7 primers were melted and hybridized (in the presence of vast excess of blocking oligonucleotides) with single-stranded plasmid library DNA prepared in vitro.

Southern hybridization of *PacI* + *Eco*RI-digested plasmid DNA from starting and normalized (with methods 1, 2-1–2-3, and 4) fetal liver/spleen libraries (Fig. 1) with several cDNA probes (including those that revealed incomplete normalization with methods 2-1–2-3, such as α-globin, β-globin and γ-globin) demonstrated the efficacy of method 4 in achieving the desired extent of normalization obtained with method 1 (cf. lanes 1–6 in Fig. 1A–D, F–H, and lanes 3–6 in Fig. 1I–K) while preserving the representation of the longest cDNAs (e.g., the longest albumin cDNA was present in the normalized library prepared with method 4, shown in lane 5 of Fig. 1D,E, but it was undetectable in the normalized library constructed with method 1, shown in lane 4; a similarly remarkable difference was revealed with the cDNA probe for H19 RNA, shown in Fig. 1G,H). Characterization of the normalized library generated with method 4 by colony hybridization with 10 cDNA probes (not shown), which occur at a wide range of frequencies in the starting library, confirmed the effectiveness of the procedure to narrow their frequencies down to within one order of magnitude (e.g., the frequencies of the cDNAs for γ-globin, α-globin, β-globin, H19 RNA, and transferrin were reduced

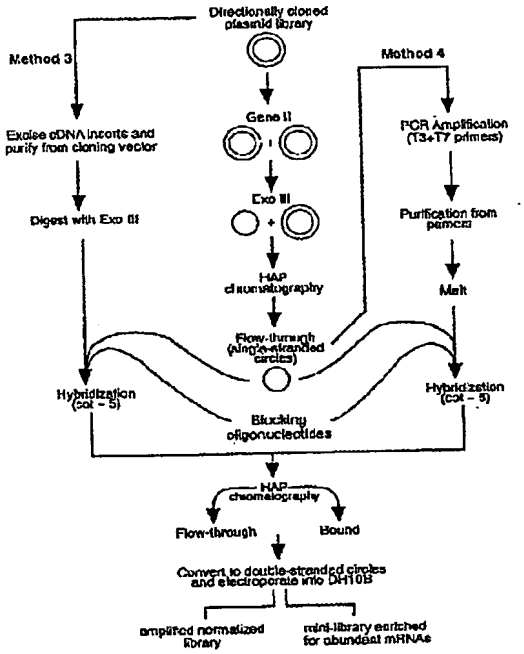

Figure 3 Diagram of the normalization methods 3 and 4. In method 3 double-stranded plasmid DNA from a starting library is digested with restriction enzymes that generate 5′ protruding ends, and the excised cDNA inserts are gel-purified from the cloning vector and digested with Exonuclease III to yield noncomplementary single-stranded fragments, each representing half of a cDNA insert. Note that the single-stranded fragments that span the 5′ half (but not the 3′ half) of the cDNA inserts are complementary to single-stranded plasmids prepared in vitro. These single-stranded DNA fragments are blocked with appropriate oligonucleotides (see Methods) and hybridized with single-stranded library DNA prepared in vitro (*middle* column). The remaining single-stranded circles are HAP-purified, converted to double-stranded plasmids, electroporated into DH10B bacteria (Life Technologies), and propagated under ampicillin selection to generate a normalized library. In method 4, single-stranded library DNA is used as template for PCR amplification with T3 and T7 primers. PCR-amplified cDNAs are purified from excess primers, melted, and hybridized with single-stranded library DNA in the presence of blocking oligonucleotides. The remaining single-stranded circles are purified by HAP chromatography, converted to double-stranded plasmids, electroporated into bacteria, and propagated under ampicillin selection to generate a normalized library.

from 9.2%, 6.4%, 3.6%, 1.8%, and <0.2% to 0.04%, 0.02%, 0.01%, 0.1% and 0.1%, respectively).

In order to assess further the ability of these normalization procedures to preferentially reduce the representation of the most abundant cDNAs, we have performed a comparative sequence analysis (not shown) of 100 clones picked randomly from the fetal liver/spleen cDNA library normalized with method 4 (14Nb2HFLS20W in Table 1; HAP-flow-through fraction in Fig. 3), and from two fetal liver/spleen mini-libraries enriched for abundant cDNAs (HAP-bound fractions in Figs. 2 and 3) obtained during HAP purification of the normalized libraries prepared according to methods 2-1 (5Nb2HFLS20W) and 4 (14Nb2HFLS20W). A number of cDNAs known to be prevalent in the starting fetal liver/spleen library (e.g., albumin, γ-globin, α-globin, β-globin, mitochondrial RNAs, and apolipoproteins A and H) were found at increased frequencies in both mini-libraries enriched for abundant cDNAs, but none of them was represented in the sample of 100 clones from the normalized library. It is noteworthy that while 47% of the sequences derived from the normalized library were not represented in the "all nonredundant" subdivision of sequences of GenBank + EMBL + DDBJ + PDB, the majority of the sequences obtained from the mini-libraries of abundant cDNAs derived from methods 2-1 and 4 (91.4% and 86.9%, respectively) did have homologous sequences in that data base. Furthermore, although 49% of the sequences derived from the normalized library had fewer than 10 homologous ESTs in the dbEST subdivision of GenBank, most of the sequences obtained from both mini-libraries had greater than 10 homologous ESTs in the dbEST data base (92.5% and 89.7%, respectively, in the HAP-bound fractions of methods 2-1 and 4).

With the ultimate goal of facilitating the ongoing process of gene discovery by large-scale sequencing of cDNA clones picked randomly from libraries, we have performed a pilot subtractive hybridization experiment to eliminate (or reduce representation of) a pool of approximately 5000 IMAGE Consortium-arrayed cDNA clones (pool no. 1, LLAM 78-90) from the normalized library from which they were derived (1NFLS in Table 1). PCR-amplified cDNA inserts from pool no. 1 were melted and hybridized, in the presence of blocking oligonucleotides, with single-stranded plasmid DNA from the 1NFLS library, prepared in vitro. The remaining single-stranded circles were purified by HAP chromatography, converted to double-stranded plasmids, electroporated into bacteria, and propagated under antibiotic selection to generate the subtracted 1NFLS-S1 library (see Fig. 4). Preliminary characterization of the 1NFLS-S1 library by Southern hybridization with 10 cDNA probes (only five are shown; see Fig. 5) known to be represented in pool no. 1 indicated clearly the effectiveness of the procedure to eliminate (or to reduce the representation of) all 11 cDNA sequences in the 1NFLS library. A BLASTN search of the dbEST division of GenBank (6/12/96) with 3' ESTs obtained from the five probes (cDNAs -1, -4, -8, -9, and -10) the hybridizations of which were not shown in Figure 5, revealed the presence of 0, 0, 1, 2, and 2 corresponding ESTs, respectively, from the 1NFLS library, thus indicating that the subtraction was successful even for cDNAs that were underrepresented in the normalized library (a total of 44,407 3' ESTs have been derived from the 1NFLS library to date). It should be noted that because of sequencing failures, some of the clones in these arrays may not yet have corresponding ESTs in the public data bases.

It is noteworthy that when we attempted to perform the same subtractive hybridization experiment using, as driver, RNA synthesized in vitro from a plasmid DNA preparation of pool no. 1, the results obtained were not satisfactory (not shown) in that subtraction could be demonstrated for some but not all tested clones (e.g, α-globin could not be subtracted effectively), similar to what we observed in normalizations with method 2-1.

DISCUSSION

As a result of an effort to improve the representation of the longest cDNAs in our normalized libraries, we have developed four different methods for normalization of directionally cloned cDNA libraries constructed in phagemid vectors, while contributing resources to the IMAGE Consortium (Lennon et al. 1996) and thereby facilitating the ongoing gene discovery and mapping programs. Approximately 87.5% of all (human) IMAGE ESTs were derived from the normalized libraries described here.

The normalization procedure (method 1) that we described previously (Soares et al. 1994) was applied for the construction of the 1NIB and 1NFLS normalized libraries, from which a total of 45,192 and 86,088 ESTs, respectively, have been derived (dbEST release 052396; http://

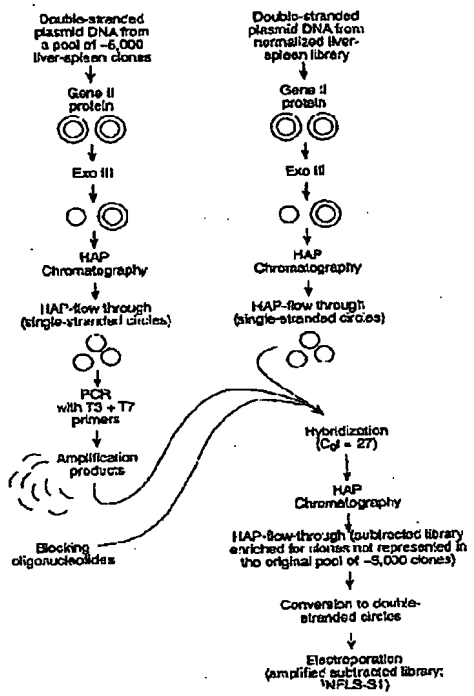

Figure 4 Diagram of the subtractive hybridization procedure used to generate the 1NFLS-S1 library. Double-stranded plasmid DNA from a pool of ~5000 IMAGE Consortium-arrayed cDNA clones derived from the 1NFLS library (pool no. 1, LLAM 78-90) was converted to single-stranded circles in vitro by the combined action of Gene II and Exonuclease III (Life Technologies). The resulting single-stranded plasmids were HAP-purified and used as a template for PCR amplification with T3 and T7 primers. PCR-amplified cDNA inserts were purified from excess primers, melted, and hybridized with single-stranded circles (prepared in vitro) from the 1NFLS library, in the presence of appropriate blocking oligonucleotides. The remaining single-stranded circles were purified by HAP chromatography, converted to double-stranded plasmids, electroporated into DH10B bacteria (Life Technologies), and propagated under ampicillin selection to generate the (1NFLS-S1) subtracted library.

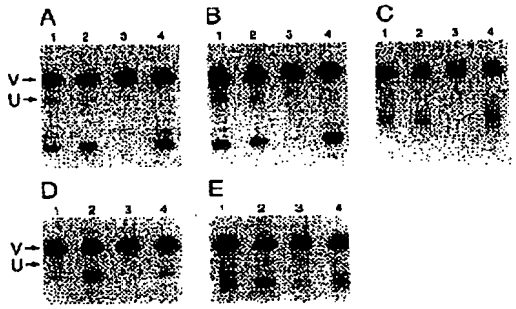

Figure 5 Characterization of the 1NFLS-S1 subtracted liver/spleen library by Southern hybridization with 5 cDNA probes. The 0.15 μg *Pac*I + *Eco*RI-digested plasmid DNA from the fetal liver/spleen library normalized with method 1 (1NFLS; lane *1*), from the pool of ~5000 IMAGE Consortium-arrayed cDNA clones derived from the 1NFLS library (pool no. 1, LLAM 78-90; lane *2*), from the subtracted library generated according to the diagram shown in Fig. 4 (1NFLS-S1; lane 3), and from the HAP-bound fraction obtained during HAP purification of the 1NFLS-S1 library (see Fig. 4) were electrophoresed, transferred to nylon membranes, and hybridized as described in the legend to Fig. 1. The following cDNA probes were used: α-globin (*A*), γ-globin (*B*), serum albumin (*C*), unknown cDNA 7 (*D*; picked randomly from pool no. 1, LLAM 78-90), and unknown cDNA 5 (*E*; picked randomly from pool no. 1, LLAM 78-90). A BLASTN search of the dbEST subdivision of Genbank with 3′ ESTs derived from cDNA 7 and cDNA 5 revealed the presence of 33 and 0 corresponding ESTs, respectively, from the 1NFLS library. All probes were contaminated intentionally with a small amount of vector DNA to enable visualization of vector bands and thus confirm that a similar amount of library DNA was loaded in all lanes. (V) vector band; (U) residual undigested plasmid.

www.ncbi.nlm.nih.gov). Data analysis (see Hillier et al., this issue) demonstrated solidly the efficacy of this approach in bringing the frequency of all clones to within a narrow range. Extensive characterization of these two libraries by Southern analysis, however, revealed that on occasion truncated clones were favored over their longest counterparts during the normalization procedure.

Because of the relatively permissive conditions used for synthesis of first-strand cDNA, priming with the *Not*I–tag–$(dT)_{18}$ oligonucleotide may occur not only at the poly(A) tail of the mRNAs but also at internal A-rich sites within the mRNAs (e.g., at Alu tails). Typically, cDNAs with 3′ truncations occur at frequencies of 10–15% in directionally cloned libraries. Truncated clones can be recognized (tentatively) as such, by the absence of a bona fide polyadenylation signal

sequence at the appropriate distance upstream from the oligo$(dA)_{18}$ tail of the cDNA.

Why may truncated cDNAs be favored over their longest counterparts during normalization by method 1? Briefly, method 1 (Soares et al. 1994) involves: (1) annealing of a single-stranded DNA preparation of a directionally cloned cDNA library with an oligo$(dT)_{18}$ primer; (2) controlled primer extension reactions in the presence of deoxynucleotides and dideoxynucleotides to generate 3′ noncoding extension products of approximately 200–300 nucleotides; (3) purification of the resulting partially double-stranded circles by HAP chromatography; (4) melting and reassociation of the HAP-purified partially double-stranded circles to a relatively low C_0t (5–10); (5) purification of the remaining single-stranded circles (normalized library) over HAP; (6) conversion of the single-stranded circles to double-stranded circles; and (7) electroporation into bacteria.

It could be anticipated that during the reassociation reaction, because truncated cDNAs occur at lower frequencies than their nontruncated counterparts, the extension products of the truncated cDNAs would more likely reanneal to the nontruncated overlapping cDNAs than to their own truncated templates. On the other hand, the extension products of the nontruncated cDNAs would most likely reassociate to their own nontruncated templates not only because they are more prevalent but also because of the low probability of there being an overlap between the short extension product of a nontruncated clone and a truncated single-stranded circle. As a result, nontruncated single-stranded circles are more likely to end up reassociated with more than one (nonoverlapping) extension product, whereas their truncated counterparts would remain single-stranded and therefore end up in the HAP flow-through fraction (normalized library).

Reasoning that this problem could be circumvented if the hybridizing fragments (1) were in excess over single-stranded circles, and (2) spanned the entire length of the cDNAs to maximize the opportunity of overlap between truncated and nontruncated clones, we devised an approach (methods 2-1 and 2-2; note that 2-2 is the same as 2-1 except that hybridization conditions were different) whereby in vitro synthesized RNA from a plasmid DNA preparation of a starting library is used as driver in hybridization ($C_0t - 5$) with the same library in the form of single-stranded circles. Indeed, these modifications improved successfully the representation of the longest cDNAs in the normalized libraries (e.g., serum albumin in the liver/spleen libraries).

However, in every library constructed with methods 2-1 and 2-2, we were able to identify cDNA clones that seemed to become normalized with much greater difficulty than others (e.g., α-globin in the 5Nb2HFLS20W liver/spleen library, and G3PD in the breast library). We interpreted these results as suggestive that not all clones might be transcribed in vitro with the same efficiency if in a mixture (i.e., in vitro transcription of plasmid DNA from an entire library), and/or secondary structures in the RNAs (or interactions between RNAs) might impair their ability to hybridize with the single-stranded circles. These hypotheses were corroborated by the observation (not shown) that relatively weak hybridization signals were observed when Northern blots of RNA transcribed in vitro from an entire plasmid library were hybridized with cDNA probes derived from those clones that could not be normalized as effectively, despite the fact that they occurred at high frequencies in the starting libraries from which the in vitro transcribed RNAs were synthesized. We did exclude the possibility that the clones that were not being normalized effectively carried deletions that prevented them from being transcribed appropriately in vitro (not shown). In fact, all clones that were tested individually for in vitro transcription yielded the expected amounts of full-length RNA. Although this problem was significantly minimized in method 2-3 (cf. lanes, 1,2 in Fig. 1A–D,F,G) the extent of normalization that was achieved was still not comparable to that obtained with method 1 (cf. lanes 2,4 in Fig. 1A–D,F,H).

The advantage of method 2-3 over methods 2-1 and 2-2 is that the RNA driver is derived from a mini-library (of relatively low complexity) enriched for abundant cDNAs rather than from the entire starting library. For this reason, higher C_0t hybridizations can be carried out to eliminate or reduce significantly the representation of the most abundant cDNAs. It should be noted, however, that method 2-3 is not a true normalization procedure, because the aim of this approach is not to equalize the frequency of all cDNA clones but rather to reduce significantly (or even to eliminate, depending on the C_0t used) the representation of the most abundant clones.

The extent to which the enrichment for abundant transcripts can be achieved in such

mini-libraries depends essentially on the C_0t used for reassociation. Calculations based on estimates of frequencies of brain mRNAs (Soares et al. 1994) indicate that the best enrichments are obtained at a $C_0t = 5–10$. If the C_0t is too low (≤1) the enrichment is only for the most prevalent (class I) mRNAs; there is no enrichment for the mRNAs of the intermediate frequency class (class II) mRNAs. On the other hand, if the C_0t is too high (≥50) the enrichment for class I transcripts starts to become less significant because of a higher representation of mRNAs of the complex class (class III). Prevalent and intermediate (classes I + II) brain mRNAs comprise 93–95% of the total cDNA population in a $C_0t = 5–10$ HAP-bound mini-library, in contrast to 62% in the starting library. Consequently, the frequency of class III transcripts in a $C_0t = 5–10$ HAP-bound mini-library is about 5.5-fold lower than that of the starting library (5–7% in the bound mini-library vs. 38% in the starting library).

Methods 3 and 4 were developed as a result of an attempt to achieve both the adequate extent of normalization obtained with method 1 and the improved representation of the longest cDNAs accomplished with methods 2-1, 2-2, and 2-3. Although more technically cumbersome, method 3 is superior to method 4 in that the DNA driver used in the hybridization is single-stranded.

Single-stranded driver in method 3 (see Fig. 3) is generated by Exonuclease III digestion of gel-purified double-stranded cDNA inserts excised from the starting library. The resulting non-complementary single-stranded fragments represent the 5′ and 3′ halves of the original cDNA inserts. The fragments that correspond to the 5′ halves of the cDNAs are complementary to single-stranded circles prepared in vitro, whereas the single-stranded fragments that correspond to the 3′ halves of the cDNA inserts are complementary to single-stranded plasmids prepared in vivo. Note that for the multiple sclerosis plaques library constructed with method 3 we used single-stranded circles prepared in vitro.

Production of single-stranded circles in vitro by the combined action of Gene II and Exonuclease III (Life Technologies), rather than in vivo by superinfection of a culture with a helper phage, is very beneficial because it circumvents the distortions that otherwise may arise as a result of the differential growth properties of clones with different size inserts. However, because the digestion with Gene II results in the conversion of most, but not all, supercoiled plasmids to relaxed circles, it becomes necessary to purify the single-stranded circles that are produced after digestion with Exonuclease III by HAP chromatography.

For construction of the normalized multiple sclerosis plaques library, the cDNA inserts were excised by double digestion of plasmid DNA from the starting library with *Not*I and *Eco*RI. The fact that one in every three clones might have an internal *Eco*RI site (an *Eco* RI site is expected to occur once every 4096 bp, and the average insert size in these libraries is of the order of 1.4 kb) should not compromise the efficiency of the procedure, because at least one of the resulting restriction fragments would be expected to be ≥200 bp (clones smaller than 400 bp are size-selected out of these libraries) and therefore be able to form hybrids that would bind quantitatively to HAP under our conditions. A disadvantage of method 3, as presented, is that only clones <2.9 kb (approximate vector size) can be excised cleanly from the vector. It is conceivable, however, that one might be able to use double-stranded cDNA fragments generated by PCR amplification with T3 and T7 primers as substrate for the Exonuclease III digestion in method 3.

Method 4 was used to generate a significant fraction of the libraries that were contributed to the IMAGE Consortium (see Table 1). It is undoubtedly the simplest and overall most advantageous of all procedures. Because the DNA driver is generated by PCR amplification of the starting (double-stranded or single-stranded, see below) plasmid library with T3 and T7 primers, the tracer (single-stranded circles) used in this hybridization may be produced in vitro or in vivo.

The extent of normalization achieved with method 4 was comparable to that obtained with method 1 with the advantage that it successfully preserved the representation of the longest cDNAs (cf. lanes 4,5 in Fig. 1). Moreover, method 4 is superior to method 1 because it does not preclude the clones derived from mRNAs with internal *Not*I sites from being represented in the normalized library. Because the starting material for the reassociation kinetics reaction in method 1 is generated by a controlled primer extension reaction with an oligo$(dT)_{18}$ primer, clones without an oligo$(dA)_{18}$ tail (derived from mRNAs with an internal *Not*I site) are not represented in the final normalized library, although they are not necessarily lost (clones without tails end up in the HAP flow-through fraction during HAP purification of the partially double-stranded circles

generated by this primer extension reaction). It should also be noted that this problem of method 1 could be circumvented by the use of an oligonucleotide complementary to flanking vector sequences [as opposed to the oligo(dT)$_{18}$] for this controlled primer extension reaction.

The potential biases introduced by PCR amplification in method 4 are minimized by the fact that (1) PCR amplification products are used in excess in these hybridizations, and (2) the size distribution of inserts in these libraries is relatively narrow (ranging typically from 0.4 to 2.5 kb).

The conditions used for hybridization greatly influenced the quality of the resulting normalized libraries constructed with method 4. This is to a great extent a consequence of the fact that we are using HAP to purify single-stranded circles, as opposed to a biotin-avidin capture system, which in our hands yielded significantly less satisfactory results (M.F. Bonaldo and M.B. Soares, unpubl.). The best results were obtained when the hybridization conditions were the most similar to the HAP conditions. We interpreted these results as suggestive of the fact that imperfect hybrids formed during hybridization may either not bind to HAP and/or may melt once in the HAP buffer.

It is noteworthy that a much superior extent of normalization was obtained with method 4 when single-stranded plasmid DNA prepared in vitro, as opposed to double-stranded plasmid DNA, was used as template for PCR amplification (not shown). These results suggest that a fraction of the double-stranded plasmids used as template for PCR amplification, presumably in the form of melted supercoiled DNA, might end up in the HAP flow-through fraction (normalized library) during purification.

It is noteworthy that cross-hybridizing diverged sequences seem to escape normalization in all of the procedures discussed above. For example, the frequency of Alu repeat-containing cDNAs (typically 10% in directionally cloned cDNA libraries) is practically the same in starting and normalized libraries. These results suggest that imperfect hybrids either do not bind to HAP under our conditions or melt once diluted in the (more stringent) HAP buffer. This is advantageous, not only because it preserves the representation of Alu-containing cDNAs that might correspond to otherwise rare mRNAs, but also, and most significant, because it minimizes the likelihood that a rare member of a gene family might be excluded from the final (normalized or subtracted) library as a result of a cross-hybridization with a more prevalent but diverged sequence.

The use of normalized libraries for large-scale gene discovery/EST programs is beneficial because it minimizes redundancies while increasing the representation of the rarer cDNAs by about threefold, on average. However, given the great extent of overlap in gene expression among different tissues, the use of normalized libraries alone is not sufficient to maintain a desirable pace of identification of novel sequences at advanced stages of such programs. For this reason, we propose that the use of subtracted libraries enriched for clones not yet identified might become increasingly advantageous. Toward this goal, we have developed a subtractive hybridization approach designed specifically for this purpose (see Fig. 4). In a pilot experiment, we were able to reduce significantly the representation of ~5000 INFLS–IMAGE Consortium clones from the INFLS library itself (see Fig. 5). With the development of appropriate clustering algorithms, the use of nonredundant sets of cDNA/gene sequences as drivers for hybridizations to generate subtractive libraries enriched for novel sequences should soon become possible, and hopefully will facilitate the isolation of all human and mouse cDNAs still awaiting identification.

METHODS

Construction of Directionally Cloned cDNA Libraries

Poly(A)$^+$ RNA was purified from total cellular RNA (except for senescent fibroblasts from which cytoplasmic RNA was isolated) using the Oligotex mRNA kit (Qiagen) according to the manufacturer's instructions, except that two rounds of purification were performed. cDNA library construction was essentially as described before (Adams et al. 1993b; Soares 1994). Typically, 1 μg poly(A)$^+$ RNA was annealed at 37°C with a twofold mass excess of a *Not*I–tag–(dT)$_{18}$ primer [or *Pac*I–tag–(dT)$_{18}$ in the case of the liver/spleen library] and reverse transcribed at 37°C with Superscript Reverse Transcriptase (Life Technologies). Alternatively poly(A)$^+$ RNA was annealed at 45°C with a fourfold mass excess of a *Not*I–tag–(dT)$_{25}$ primer and reverse transcribed at 45°C. The tag is a sequence of 2–6 nucleotides that is unique for each library and thus serves as an identifier (see Table 1). With the exception of infant brain, fetal liver/spleen and term placenta, all other first-strand cDNA syntheses were primed with the following oligonucleotide: TGTTACCAATCTGAAGTGGGAGCGGCCGC–tag–(dT)$_{18 \text{ or } 25}$. The oligonucleotide AACTGGAAGAATTCGCGGCCGCAGGAA(dT)$_{18}$ (Pharmacia) was used to prime both infant brain and term placenta first-strand cDNA syntheses. The oligonucleotide AACTGGAAGAATTAATTAAAGATCT(dT)$_{18}$ was used to prime the synthesis of first-

strand fetal liver/spleen cDNA. Double-stranded cDNAs were size-selected by gel filtration over a long (64-cm) and narrow (0.2-cm diameter) Bio-Gel A-50m (Bio-Rad, 100–200 mesh) column, and ligated to a 500- to 1000-fold molar excess of adapters. Infant brain cDNAs were ligated to *Hin*dIII adapters, digested with *Not*I, size selected over a second Bio-Gel column, and cloned directionally into the *Not*I and *Hin*dIII sites of the Lafmid BA vector (Soares et al. 1994). Fetal liver/spleen cDNAs were ligated to *Eco*RI adapters (Pharmacia), size-selected as above, digested with *Pac*I and cloned directionally into the *Pac*I and *Eco*RI sites of the pT7T3–*Pac* vector. All other cDNAs were ligated to *Eco*RI adapters (Pharmacia), size-selected as above, digested with *Not*I and cloned directionally into the *Not*I and *Eco*RI sites of the pT7T3–*Pac* vector. pT7T3–*Pac* is essentially the same as pT7T318D (Pharmacia) with a modified polylinker. Figure 6 shows the sequence of the pT7T3–*Pac* polylinker and flanking sequences.

Production of Purified Covalently Closed Single-stranded Library DNA in Vitro

Double-stranded phagemid DNA was converted to single-stranded circles by the combined action of Gene II (phage f1 endonuclease) and *Escherichia coli* Exonuclease III enzymes, as per the manufacturer's instructions (Life Technologies; cat. no. 10356-020). The resulting single-stranded circular DNA was purified from the remaining double-stranded plasmids by HAP chromatography (Bio-Rad) as described previously (Soares et al. 1994). The replication initiator protein of bacteriophage f1 (Gene II) is a site-specific endonuclease that binds to the f1 origin in phagemid vectors and nicks the viral strand of the supercoiled DNA. The nicked strand is then digested from its 3′ end with Exonuclease III (Hoheisel 1993) to generate single-stranded circles. Purification of the resulting single-stranded circles over HAP is necessary because the conversion of supercoiled to relaxed plasmids by Gene II is never complete. The Gene II reaction was performed for 1 hr at 30°C and contained typically 4 μg supercoiled plasmid library DNA, 1 μl Gene II (Life Technologies), and 2 μl 10× Gene II buffer (Life Technologies) in a total volume of 20 μl. The Gene II protein was heat inactivated for 5 min at 65°C; the reaction mixture was chilled on ice; 2 μl Exonuclease III (Life Technologies, Cat. No. 18013-011, 65 units/μl) was added; and the reaction was incubated for 30 min at 37°C. Gene II and Exonuclease III were then digested with Proteinase K (Boehringer Mannheim) for 15 min at 50°C in a 100-μl reaction containing 10 mM Tris (pH 7.8), 5 mM ethylenediamine tetraacetic acid (EDTA), 0.5% SDS, and 136 μg Proteinase K. After extraction with equal volume of phenol–chloroform–isoamyl alcohol (25:24:1), library DNA was ethanol-precipitated and digested with *Pvu*II for 2 hr at 37°C. This was done to convert the remaining supercoiled plasmids into linear DNA molecules and thereby improve their bindability to HAP under our conditions. Note that *Pvu*II does not cleave single-stranded circles and that there are two *Pvu*II sites in the vector. The reaction was diluted with 2 ml loading buffer [0.12 M sodium phosphate buffer (pH6.8), 10 mM EDTA, and 1% SDS] and purified by HAP chromatography at 60°C, using a column pre-equilibrated with the same buffer (1-ml bed vol.; 0.4 g of HAP). After a 6-ml wash with loading buffer, this volume was combined with the flow-through fraction, and the sample was extracted twice with water-saturated 2-butanol, once with dry 2-butanol, and once with water-saturated ether (3 vols. per extraction). Residual ether was blown off by vacuum and the sample was desalted by passage through a Nensorb column (DuPont/NEN) according to the manufacturer's specifications, concentrated down to ~0.35 ml and ethanol-precipitated. Note that Gene II–Exonuclease III prepared single-stranded DNA is in the opposite polarity to single-stranded DNA generated by in vivo phagemid production.

Production of Purified Covalently Closed Single-stranded Library DNA in Vivo

Plasmid DNA from the starting library was electroporated into *E. coli* DH5αF′ bacteria, and the culture was grown under ampicillin selection at 37°C to an OD_{600} of 0.2, superinfected with a 10- to 20-fold excess of the helper phage M13KO7 (Pharmacia), and harvested after 4 hr for preparation of single-stranded plasmids, as described (Vieira and Messing 1987).

Conversion of Single-stranded Circles to Double-stranded Plasmids

Single-stranded circles (<50 ng) were ethanol-precipitated and resuspended in 11 μl water. Then 4 μl 5× Sequenase buffer (USB) and 1 μl primer (1 μg) were added and the mixture was incubated at 65°C for 5 min and then at 37°C for 3 min. Then 1 μl Sequenase version 2.0 (USB), 1 μl 0.1 M dithiothreitol (DTT), and 2 μl mixed dNTP stock (a solution containing each deoxynucleotide at a final concentration of 10 mM) were added, and the reaction was incubated at 37°C for 30 min. The total volume was taken up to 100 μl with 10 mM Tris (pH8.0) and 1 mM EDTA (TE) and the reaction was extracted once with phenol–chloroform–isoamyl alcohol (25:24:1). Plasmid DNA was ethanol-precipitated and dissolved in 3 μl TE. The following oligonucleotides were used for this primer extension reaction: (1) M13 Reverse Sequencing Primer (5′-AGCGGATAACAATTTCACACAGGA-3′), which is complementary to single-stranded prepared in vitro,

5′-cacccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacaggaaacagctatg
M13 Reverse Sequencing Primer

acatgattacgaatttaatacgactcactatagggaatttGGCCCTCGAGGCCAAGAATTCCCGACTACGTA
T7 Promoter *Sfi*I *Eco*RI *Sna*BI

GTCGGGGATCCGTCTTAATTAAGCGGCCGCAAGCTTattccctttagtgagggttaattttagcttggcac
*Bam*HI *Pac*I *Not*I *Hin*dIII T3 Promoter

tggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcag-3′.
M13 Sequencing Primer

Figure 6 Sequence of the pT7T3–*Pac* polylinker (uppercase) and flanking sequences (lowercase).

and (2) Oligo-Amp (5′-GACTGGTGAGTACTCAAC-CAAGTC-3′), which is complementary to the ampicillin resistance gene of single-stranded pT7T3-Pac or Lafmid BA plasmids prepared in vivo.

In Vitro Synthesis of Library RNA

Some 2–5 μg of double-stranded plasmid DNA from either the starting library (see methods 2-1 and 2-2 below) or the mini-library of abundant cDNAs (see method 2-3 below) was linearized with either *Pac*I (NEB) or *Not*I (NEB) and used as a template for synthesis of RNA with RiboMax Large Scale RNA Production Systems T7 or T3 (Promega), according to the manufacturer's instructions. After treatment with ribonuclease-free DNAse I (Promega), to digest away the plasmid DNA template, the RNA was used for hybridization as described below. It should be noted that RNA synthesized with T7 RNA Polymerase is in the message-like orientation and is complementary to the single-stranded circles produced in vitro. On the other hand, RNA synthesized with T3 RNA Polymerase is in the antimessage orientation and it is complementary to single-stranded circles produced in vivo.

Normalization Method 1

The procedure used for construction of the normalized human infant brain (1NIB) library (here designated as method 1) has been described previously (Soares et al. 1994). Method 1, with minor modifications, was also applied to construct the normalized human fetal liver/spleen cDNA library (1NFLS). To synthesize a partial second strand of about 200 nt by limited extension, a 100 μl reaction mixture containing 5 μl 0.5 μg/μl *Pvu*II-digested, HAP- and gel-purified single-stranded plasmid DNA from the fetal liver/spleen starting library, 7 μl 10 ng/μl oligo $(dT)_{12-18}$ (Pharmacia), 10 μl 10× Primer Extension Buffer [0.3 M Tris (pH 7.5), 0.5 M NaCl, and 0.15 M $MgCl_2$], 10 μl 0.1 M DTT, 10 μl mixed dNTP stock, 25 μl mixed ddNTP stock (a solution containing each dideoxy A, C, and G at a final concentration of 25 mM), 5 μl 800 Ci/mmole [α-^{32}P]dCTP, and 20.5 μl water was incubated at 60°C for 5 min, at 50°C for 15 min, and at 37°C for 2 min. Then 7.5 μl 5 units/μl Klenow enzyme (USB) was added, and the reaction was incubated at 37°C for 30 min. The reaction was extracted with phenol–chloroform–isoamyl alcohol (25:24:1), 5 μg melted and sheared salmon sperm DNA was added, and the partially double-stranded plasmids were purified from the remaining single-stranded circles (unprimed molecules, as well as clones derived from mRNAs with an internal *Pac*I site that therefore do not contain an oligo(dA) tail at the 3′ end) by HAP chromatography. The HAP-bound fraction containing the partially double-stranded plasmids was eluted with 6 ml 0.4 M sodium phosphate buffer (pH 6.8), 10 m M EDTA, and 1% SDS, and plasmid DNA was desalted as described before (Soares et al. 1994) and ethanol-precipitated. The DNA (173 ng) was resuspended in 2.5 μl deionized formamide and melted at 80°C for 3 min under 10 μl mineral oil. Then 1 μl of 5 μg/μl oligo $(dT)_{12-18}$ (used to block the tails) was added, and the mixture was heated at 80°C for 1 min. Then 0.5 μl 5 M NaCl, 0.5 μl 10× TE, and 0.5 μl water were added, and the reassociation reaction was incubated at 42°C for 0.6 hr (calculated $C_0t = 0.5$). The remaining single-stranded circles were purified over HAP (flow-through fraction) and subjected subsequently to a second cycle of the normalization procedure as described above, except that reassociation was conducted for 24 hr (calculated $C_0t = 20$). The remaining single-stranded circles (normalized library; 1NFLS) were purified over HAP, converted to double-stranded plasmids, electroporated into DH10B bacteria, and propagated under ampicillin selection.

Normalization Methods 2-1, 2-2, and 2-3

Method 2 is a reassociation kinetics-based approach involving hybridization of in vitro synthesized RNA (the driver) derived either from the entire library (methods 2-1 and 2-2; see Fig. 2) or from a mini-library enriched for abundant cDNAs (method 2-3; see Fig. 2), with the whole starting library in the form of single-stranded circles (the tracer). The remaining single-stranded circles (normalized library) are purified by HAP chromatography (HAP flow-through fraction), converted to double-stranded plasmids for improvement of electroporation efficiency, electroporated into DH10B bacteria (Life Technologies), and propagated under ampicillin selection. A number of normalized cDNA libraries were constructed with these methods using single-stranded plasmids prepared both in vivo and in vitro (see Table 1). In all three variants, the driver was first pre-annealed with a pair of oligonucleotides to block both 5′ and 3′ vector sequences as follows: 0.5 μl (10 μg) of each oligonucleotide, 1 μl RNA (5.0 μg in methods 2-1 and 2-3; 0.5 μg in method 2-2), and 4.0 μl deionized formamide were heated for 3 min at 80°C under 10 μl mineral oil and quickly chilled on ice. Then 0.8 μl 10× hybridization buffer [0.4 M Pipes (pH 6.4), 4 M NaCl, and 10 mM EDTA in methods 2-1 and 2-3; 0.4 M Pipes (pH 6.4), 1.2 M NaCl, 10 mM EDTA, and 1% SDS in method 2-2), 0.5 μl RNAsin (Boehringer Mannnheim), and 0.7 μl water were added and the mixture (total volume, 8 μl) was incubated overnight at 42°C (methods 2-1 and 2-3) or 30°C (method 2-2). In another tube, 2.5 μl (50 ng) single-stranded library DNA in deionized formamide was heated for 3 min at 80°C under mineral oil; 0.5 μl 10× hybridization buffer and 2.0 μl water were added; and the mixture was transferred to the tube containing the preannealed RNA. Hybridization (13-μl reaction) was performed at 42°C (method 2-1: C_0t = 5–10; method 2-3: C_0t = 100–200) or at 30°C (method 2-2: C_0t = 5–10). The driver, rather than the tracer, was blocked because otherwise the latter would, to some extent, bind to HAP during purification. The plasmid mini-library enriched for abundant cDNAs that served as a template for the synthesis of RNA used as driver in method 2-3 was prepared from the HAP-bound fraction obtained during purification of the normalized library in method 2-1. Different pairs of blocking oligonucleotides were used, depending on whether the RNA was synthesized with T3 or T7 RNA polymerases. To block RNA synthesized with T3 RNA polymerase, which was used in hybridizations with single-stranded plasmids prepared in vivo we used: 5′-$_{19}$AGGGCGGCCGCAAGCTTATTCCCTTTAGT-GAGGGTTAAT-3′ (this oligonucleotide was used to block 5′ vector sequences of all but the human fetal liver/spleen library RNA), and 5′-$_{19}$AGATCTTTAATTAAGCGGCCG-CAAGCTTATTCCCTTTAGTGAGGGTTAAT-3′ (this oligonucleotide was used to block 5′ vector sequences of the

human fetal liver/spleen library RNA), and 5′-AGGCCAAGAATTCGGCACGAG-3′ (this oligonucleotide was used to block 3′ vector sequences). To block RNA synthesized with T7 RNA polymerase, which was used in hybridizations with single-stranded plasmids prepared in vitro we used: 5′-CCTCGTGCCGAATTCTTGGCCTCGAGGGCCAAATTCCC-3′ (this oligonucleotide was used to block 5′ vector sequences). The oligonucleotide used to prime the synthesis of first-strand cDNA was also used to block 3′ vector sequences.

Normalization Method 3

Method 3, used to generate the normalized library from multiple sclerosis plaques (2NbHMSP), is a reassociation-kinetics based approach involving hybridization (C_0t = 20–25) of a 20-fold excess of Exonuclease III-digested cDNA inserts excised from a plasmid DNA preparation of the starting library with the library itself in the form of single-stranded circles, followed by HAP-purification of the remaining single-stranded plasmids, conversion to double-strands, and electroporation into bacteria. Some 5 μg double-stranded plasmid DNA from the starting library was doubly digested with *Not*I and *Eco*RI; the excised cDNA inserts were separated from the cloning vector by agarose gel electrophoresis; and the DNA was purified using beta-agarase (NEB) according to the manufacturer's instructions. Then 0.6 μg gel-purified double-stranded cDNA inserts in 47.5 μl TE was digested with Exonuclease III at 37°C for 30 min in a 60-μl reaction containing 6 μl 10× Exonuclease III buffer [0.5 M Tris (pH 8.0) and 50 mM $MgCl_2$)], 0.6 μl 0.1 M DTT, 2.9 μl water, and 3 μl of 65 units/μl Exonuclease III (Life Technologies). The Exonuclease was then digested with 136 μg Proteinase K (Boehringer Mannheim) at 50°C for 15 min in a 100-μl reaction containing 10 mM Tris (pH 7.8), 5 mM EDTA, and 0.5% SDS. After two extractions with phenol–chloroform–isoamyl alcohol (25:24:1), the resulting noncomplementary single-stranded DNA (total amount ~0.3 μg) was ethanol-precipitated and resuspended in 1 μl TE. A 5-μl hybridization reaction was then set up as follows: 1 μl Exonuclease III-digested cDNA inserts (an estimated amount of 150 ng of single-stranded DNA) and 50 ng single-stranded plasmid DNA from the starting multiple sclerosis plaques library (prepared in vitro) in 2.5 μl deionized formamide were mixed and heated at 80°C for 3 min under 10 μl mineral oil. Then 0.5 μl (10 μg) of a blocking oligonucleotide (5′-CCTCGTGCCGAATTCTTGGCCTCGAGGGCCAAATTCCCTATAGTGAGTCGTATTA-3′), 0.5 μl 5 M NaCl, and 0.5 μl 10× TE were added, and the mixture was incubated at 42°C for 41 hr (calculated C_0t of 23). The remaining single-stranded plasmids were purified by HAP chromatography, converted to double-stranded plasmids, and electroporated into DH10B bacteria (Life Technologies) as described above.

Normalization Method 4

This is a reassociation-kinetics-based approach involving hybridization of a 20-fold excess of cDNA inserts generated by PCR with the library itself in the form of single-stranded circles, followed by HAP purification of the remaining single-stranded plasmids, conversion to double-strands, electroporation into DH10B bacteria, and amplification under ampicillin selection. PCR amplification of cDNA inserts was performed using the Expand High Fidelity PCR System (Boehringer Mannheim) according to the manufacturer's instructions. This PCR system is composed of an enzyme mixture containing thermostable Taq DNA and Pwo DNA polymerases (Barnes 1994). An amount of 1 μl (2.5–5.0 ng) DNA template [double-stranded plasmids (fetal lung, parathyroid adenoma, senescent fibroblasts) or single-stranded circles prepared in vitro (fetal heart, 14Nb2HFLS20W-fetal liver/spleen, and all mouse, rat, and schistosome libraries listed in Table 1)] was mixed with 2 μl dNTP stock (the final concentration of each dNTP in the reaction is 200 μM), 5 μl of a 20-μM solution of T7 Primer (5′-TAATACGACTCACTATAGGG-3′), 5 μl of a 20-μM solution of T3 Primer (5′-ATTAACCCTCACTAAAGGGA-3′), 10 μl 10× 1 Expand High Fidelity buffer, 0.75 μl Expand High Fidelity enzyme mix (2.6 units), and 76.25 μl water. Then 50 μl mineral oil was added and the reaction mixture was subjected to the following amplification cycle conditions in a Perkin Elmer Thermocycler: 7 min while ramping up from room temperature to 94°C; 20 cycles of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C, and 7 min at 72°C. PCR-amplified fragments were purified using the High Pure PCR Product Purification Kit (Boehringer Mannheim) as instructed by the manufacturer. The purified PCR product was ethanol-precipitated and dissolved in 5 μl TE. Then 1.5 μl (0.5 μg) PCR products was mixed with 5 μl (50 ng) library DNA (single-stranded circles prepared in vitro) in deionized formamide, 0.5 μl (10 μg) 5′ blocking oligo AV-1 (5′-CCTCGTGCCGAATTCTTGGCCTCGAGGGCCAAATTCCCTATAGTGAGTCGTATTA-3′), 0.5 μl (10 μg) 3′ blocking oligo AR (5′-ATTAACCCTCACTAAAGGGAATAAGCTTGCGGCCGCT$_{20}$-3′; used for all but the fetal liver/spleen library), or alternatively, (0.5 μl (10 μg) 3′ blocking oligo AV-2 (5′-ATTAACCCTCACTAAAGGGAATAAGCTTGCGGCCGCTTAATTAAAGATCT$_{19}$-3′; used only for the fetal liver/spleen library), and this mixture was heated at 80°C for 3 min under 10 μl of mineral oil. Then 1 μl 10× buffer-A [1.2 M NaCl, 0.1 M Tris (pH 8.0), and 50 mM EDTA; used for fetal lung, fetal heart, parathyroid adenoma, senescent fibroblasts, and 19.5-days postconception (dpc) mouse embryo] or, alternatively, 1 μl 10× buffer-B [1.2 M NaCl, 0.1 M Tris (pH 8.0), 50 mM EDTA, and 10% SDS; used for 14Nb2HFLS20W-fetal liver/spleen, 17.5-dpc mouse embryo, 13.5- to 14.5-dpc mouse embryo, rat heart, rat kidney, and 8-week schistosome], and 1.5 μl water were added, and the hybridization was performed at 30°C for 24 hr (calculated C_0t ~ 5). The remaining single-stranded circles were purified by HAP chromatography, converted to double-strands, and electroporated into DH10B (Life Technologies) bacteria, as described above.

Subtractive Hybridization

Double-stranded plasmid DNA from a pool of 4992 clones grown individually in 384 well plates (IMAGE Consortium plates LLAM 78-90, identification nos. 66696–67079 and 108168–112775) derived from the normalized fetal liver/spleen library (1NFLS) was prepared using the Qiagen Midi-prep kit according to the manufacturer's instructions, and converted to single-stranded circles in vitro, as described above. Single-stranded circles were purified by

HAP chromatography and used as a template for PCR amplification with T7 and T3 primers, as described above. An amount of 1.5 μg of PCR-amplified cDNA inserts from the LLAM 78-90 pool (in 4 μl deionized formamide) was mixed with 50 ng of single-stranded circles from the 1NFLS library (in 2 μl deionized formamide), 2.1 μl (42 μg) 5′ blocking oligo AV-1, and 2.1 μl (42 μg) 3′ blocking oligo AV-2. Then 10 μl mineral oil was added, and the mixture was heated at 80°C for 3 min. Then 1.2 μl 10× buffer-B and 0.6 μl water were added, and the hybridization was performed at 30°C for 48 hr (calculated $C_0t = 27$). The remaining single-stranded circles were purified over HAP, converted to double-strands, electroporated into DH10B bacteria, and propagated under ampicillin selection to generate the subtracted liver/spleen library (1NFLS-S1). HAP-bound DNA was also processed and purified for use in control experiments.

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REFERENCES

Adams, M.D., J.M. Kelley, J.D. Gocayne, M. Dubnick, M.H. Polymeropoulos, H. Xiao, C.R. Merril, A. Wu, B. Olde, R.F. Moreno, A.R. Kerlavage, W.R. McCombie, and J. Craig Venter. 1991. Complementary DNA sequencing: Expressed sequence tags and Human Genome Project. *Science* **252:** 1651–1656.

Adams, M.D, M. Dubnick, A.R. Kerlavage, R. Moreno, J.M. Kelley, T.R. Utterback, J.W. Nagle, C. Fields, and J. Craig Venter. 1992. Sequence identification of 2,375 human brain genes. *Nature* **355:** 632–634.

Adams, M.D., A.R. Kerlavage, C. Fields, and J.C. Venter. 1993a. 3,400 new expressed sequence tags identify diversity of transcripts in human brain. *Nature Genet.* **4:** 256–267.

Adams, M.D., M.B. Soares, A.R. Kerlavage, C. Fields, and J.C. Venter. 1993b. Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library. *Nature Genet.* **4:** 373–380.

Adams, M.D., A.R. Kerlavage, R.D. Fleischmann, R.A. Fuldner, C.J. Bult, N.H. Lee, E.F. Kirkness, K.G. Weinstock, J.D. Gocayne, O. White, et al. 1995. Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. *Nature* **377:** 3–174.

Altschul, S.F., W. Gish, W. Miller, E. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.

Barnes, W.M. 1994. PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. *Proc. Natl. Acad. Sci.* **91:** 2216–2220.

Berry, R., T.J. Stevens, N.A. Walter, A.S. Wilcox, T. Rubano, J.A. Hopkins, J. Weber, R. Goold, M.B. Soares, and J.M. Sikela. 1995. Gene-based sequence-tagged-sites (STSs) as the basis for a human gene map. *Nature Genet.* **10:** 415–423.

Bishop, J.O., J.G. Morton, M. Rosbash, and M. Richardson. 1974. Three abundance classes in HeLa cell messenger RNA. *Nature* **250:** 199–204.

Davidson, E.H. and R.J. Britten. 1979. Regulation of gene expression: Possible role of repetitive sequences. *Science* **204:** 1052–1059.

Hillier, L., G. Lennon, M. Becker, M. Bonaldo, B. Chiapelli, S. Chissoe, N. Dietrich, T. DuBuque, A. Favello, W. Gish, et al. 1996. Generation and analysis of 280,000 human expressed sequence tags. *Genome Res.* (this issue).

Hoheisel, J.D. 1993. On the activities of Escherichia coli exonuclease III. *Anal. Biochem.* **209:** 238–246.

Houlgatte, R., R. Mariage-Samson, S. Duprat, A. Tessier, S. Bentolila, B. Lamy, and C. Auffray. 1995. The Genexpress Index: A resource for gene discovery and genic map of the human genome. *Genome Res.* **5:** 272–304.

Johnston, S., J.H. Lee, and D.S. Ray. 1985. High-level expression of M13 gene II protein from an inducible polycistronic messenger RNA. *Gene* **34:** 137–145.

Khan, A.S., A.S. Wilcox, M.H. Polymeropoulos, J.A. Hopkins, T.J. Stevens, M. Robinson, A.K. Orpana, and J.M. Sikela. 1992. Single pass sequencing and physical and genetic mapping of human brain cDNAs. *Nature Genet.* **2:** 180–185.

Lennon, G.G., C. Auffray, M. Polymeropoulos, and M.B. Soares. 1996. The I.M.A.G.E. Consortium: An integrated molecular analysis of genomes and their expression. *Genomics* **33:** 151–152.

Combie, W.R., M.D. Adams, J.M. Kelley, M.G. FitzGerald, T.R Utterback, M. Khan, M. Dubnick, A.R. Kerlavage, J.C. Venter, and C. Fields. 1992. *Caenorhabditis elegans* expressed sequence tags identify gene families and potential disease gene homologues. *Nature Genet.* **1:** 124–131.

Okubo, K., N. Hori, R. Matoba, T. Niiyama, A. Fukushima, Y. Kojima, and K. Matsubara. 1992. Large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression. *Nature Genet.* **2:** 173–179.

Rasched, I. and E Oberer. 1986. Ff coliphages: Structural and functional relationships. *Microbiol. Rev.* **50:** 401–427.

Soares, M.B. 1994. Construction of directionally cloned cDNA libraries in phagemid vectors. In *Automated DNA sequencing and anlaysis* (ed. M.D. Adams, C. Fields, and J.C. Venter), pp. 110–114. Academic Press, New York, NY.

Soares, M.B., M.F. Bonaldo, P. Jelene, L. Su, L. Lawton, and A. Efstratiadis. 1994. Construction and characterization of a normalized cDNA library. *Proc. Natl. Acad. Sci.* **91:** 9228–9232.

Vieira, J. and J. Messing. 1987. Production of single-stranded plasmid DNA. *Methods Enzymol.* **153:** 3–11.

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